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(S4) Title: FATTY ACID ELONGASES			
(57) Abstract			
<p>Nucleic acids are disclosed that encode fatty acid β-keto acyl synthases from plants. Such synthases are effective for producing very long chain fatty acids (VLCFA), e.g., C22 to C26, preferentially saturated but also monounsaturated. Also disclosed are polypeptides encoded by such nucleic acids. Transgenic plants expressing these polypeptides exhibit altered levels of VLCFA in one or more tissues, such as seeds or leaves.</p>			

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FATTY ACID ELONGASES

Field of the Invention

5 This invention relates to fatty acid elongase complexes and nucleic acids encoding elongase proteins. More particularly, the invention relates to nucleic acids encoding β -keto acyl synthase proteins that are effective for producing very long chain fatty acids, polypeptides
10 produced from such nucleic acids and transgenic plants expressing such nucleic acids.

Background of the Invention

Plants are known to synthesize very long chain fatty acids (VLCFAs). VLCFAs are saturated or
15 unsaturated monocarboxylic acids with an unbranched even-numbered carbon chain that is greater than 18 carbons in length. Many VLCFAs are 20-32 carbons in length, but VLCFAs can be up to 60 carbons in length. Important VLCFAs include erucic acid (22:1, i.e., a 22 carbon chain
20 with one double bond), nervonic acid (24:1), behenic acid (22:0), and arachidic acid (20:0).

Plant seeds accumulate mostly 16- and 18-carbon fatty acids. VLCFAs are not desirable in edible oils. Oilseeds of the Cruciferae (e.g., rapeseed) and a few
25 other plants, however, accumulate C20 and C22 fatty acids (FAs). Although plant breeders have developed rapeseed lines that have low levels of VLCFAs for edible oil purposes, even lower levels would be desirable. On the other hand, vegetable oils having elevated levels of
30 VLCFAs are desirable for certain industrial uses, including uses as lubricants, fuels and as a feedstock for plastics, pharmaceuticals and cosmetics.

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The biosynthesis of saturated fatty acids up to an 18-carbon chain occurs in the chloroplast. C2 units from acyl thioesters are linked sequentially, beginning with the condensation of acetyl Coenzyme A (CoA) and malonyl 5 acyl carrier protein (ACP) to form a C4 acyl fatty acid. This condensation reaction is catalyzed by a β -ketoacyl synthase III (KASIII). β -ketoacyl moieties are also referred to as 3-ketoacyl moieties.

The enzyme β -ketoacyl synthase I (KASI) is 10 involved in the addition of C2 groups to form the C6 to C16 saturated fatty acids. KASI catalyzes the stepwise condensation of a fatty acyl moiety (C4 to C14) with malonyl-ACP to produce a 3-ketoacyl-ACP product that is 2 carbons longer than the substrate. The last condensation 15 reaction in the chloroplast, converting C16 to C18, is catalyzed by β -ketoacyl synthase II (KASII).

Each elongation cycle involves three additional enzymatic steps in addition to the condensation reaction as discussed above. Briefly, the β -ketoacyl condensation 20 product is reduced to β -hydroxyacyl-ACP, dehydrated to the enoyl-ACP, and finally reduced to a fully reduced acyl-ACP. The fully reduced fatty acyl-ACP reaction product then serves as the substrate for the next cycle of elongation.

25 The C18 saturated fatty acid (stearic acid, 18:0) can be transported out of the chloroplast and converted to the monounsaturate C18:1 (oleic acid), and the polyunsaturates C18:2 (linoleic acid) and C18:3 (α -linolenic acid). C18:0 and C18:1 can also be elongated 30 outside the chloroplast to form VLCFAs. The formation of VLCFAs involves the sequential condensation of two carbon groups from malonyl CoA with a C18:0 or C18:1 fatty acid substrate. Elongation of fatty acids longer than 18 carbons depends on the activity of a fatty acid elongase 35 complex to carry out four separate enzyme reactions

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similar to those described above for fatty acid synthesis in the chloroplast. Fehling, Biochem. Biophys. Acta 1082:239-246 (1991). In plants, elongase complexes are distinct from fatty acid synthases since elongases are 5 extraplastidial and membrane bound.

Mutations have been identified in an *Arabidopsis* gene associated with fatty acid elongation. This gene, designated the *FAE1* gene, is involved in the condensation step of an elongation cycle. See, WO 96/13582, 10 incorporated herein by reference. Plants carrying a mutation in *FAE1* have significant decreases in the levels of VLCFAs in seeds. Genes associated with wax biosynthesis in jojoba have also been cloned and sequenced (WO 95/15387, incorporated herein by 15 reference).

Very long chain fatty acids are key components of many biologically important compounds in animals, plants, and microorganisms. For example, in animals, the VLCFA arachidonic acid is a precursor to many prostaglandins.

20 In plants VLCFAs are major constituents of triacylglycerols in many seed oils, are essential precursors for cuticular wax production, and are utilized in the synthesis of glycosylceramides, an important component of the plasma membrane.

25 Obtaining detailed information on the biochemistry of KAS enzymes has been hampered by the difficulties encountered when purifying membrane bound enzymes.

Although elongase activities have been partially purified from a number of sources, or studied using cell

30 fractions, the elucidation of the biochemistry of elongase complexes has been hampered by the complexity of the membrane fractions used as the enzyme source. For example, until recently, it was unclear as to whether plant elongase complexes were composed of a

35 multifunctional polypeptide similar to the FAS found in

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animals and yeast, or if the complexes existed as discrete and dissociable enzymes similar to the FAS of plants and bacteria. Partial purification of an elongase KAS, immunoblot identification of the hydroxy acyl dehydrase, and the recent cloning of a KAS gene (*FAE1*) suggest that the enzyme activities of elongase complexes exist on individual enzymes.

Summary of the Invention

The invention disclosed herein relates to an isolated polynucleotide selected from one of the following: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; an RNA analog of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15; and a polynucleotide having a nucleic acid sequence complementary to one of the above. The polynucleotide can also be a nucleic acid fragment of one of the above sequences that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

Also disclosed herein is an isolated polypeptide that has an amino acid sequence substantially identical to one of the following: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14. Also disclosed are isolated polynucleotides encoding polypeptides substantially identical in their amino acid sequence to: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

The invention also relates to a transgenic plant containing a nucleic acid construct. The nucleic acid construct comprises a polynucleotide described above. The construct further comprises a regulatory element

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operably linked to the polynucleotide. The regulatory element may a tissue-specific promoter, for example, an epidermal cell-specific promoter or a seed-specific promoter. The regulatory element may be operably linked 5 to the polynucleotide in sense or antisense orientation. The plant has altered levels of very long chain fatty acids in tissues where the polynucleotide is expressed, compared to a parental plant lacking the nucleic acid construct.

10 A method is disclosed for altering the levels of very long chain fatty acids in a plant. The method comprises the steps of creating a nucleic acid construct and introducing the construct into the plant. The construct includes a polynucleotide selected from one of 15 the following: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; an RNA analog of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15; and a polynucleotide having a nucleic acid sequence complementary to one of the above. The polynucleotide 20 can also be a nucleic acid fragment of one of the above that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ 25 ID NO:14. The polynucleotide is effective for altering the levels of very long chain fatty acids in the plant.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

30

Brief Description of the Drawings

Figure 1 shows the time course of *in vitro* VLCFA synthesis by FAE1 expressed in yeast, with 3 different acyl-CoA substrates.

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Figure 2 shows the rates of *in vitro* VLCFA synthesis and the VLCFA profiles of FAE1 expressed in yeast, with 3 different acyl-CoA substrates.

5 Figure 3 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL1 polynucleotide (SEQ ID NO:1).

Figure 4 shows the deduced amino acid sequence (SEQ ID NO:2) for the EL1 coding sequence of Figure 3.

10 Figure 5 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL2 polynucleotide (SEQ ID NO:3).

Figure 6 shows the deduced amino acid sequence (SEQ ID NO:4) for the EL2 coding sequence of Figure 5.

15 Figure 7 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL3 polynucleotide (SEQ ID NO:5).

Figure 8 shows the deduced amino acid sequence (SEQ ID NO:6) for the EL3 coding sequence of Figure 7.

20 Figure 9 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL4 polynucleotide (SEQ ID NO:7).

Figure 10 shows the deduced amino acid sequence (SEQ ID NO:8) for the EL4 coding sequence of Figure 9.

25 Figure 11 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL5 polynucleotide (SEQ ID NO:9).

Figure 12 shows the deduced amino acid sequence (SEQ ID NO:10) for the EL5 coding sequence of Figure 11.

30 Figure 13 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL6 polynucleotide (SEQ ID NO:11).

Figure 14 shows the deduced amino acid sequence (SEQ ID NO:12) for the EL6 coding sequence of Figure 13.

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Figure 15 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL7 polynucleotide (SEQ ID NO:13).

Figure 16 shows the deduced amino acid sequence 5 (SEQ ID NO:14) for the EL7 coding sequence of Figure 15.

Description of the Preferred Embodiments

The present invention comprises isolated nucleic acids (polynucleotides) that encode polypeptides having β -ketoacyl synthase activity. The novel polynucleotides 10 and polypeptides of the invention are involved in the synthesis of very long chain fatty acids and are useful for modulating the total amounts of such fatty acids and the specific VLCFA profile in plants.

A polynucleotide of the invention may be in the 15 form of RNA or in the form of DNA, including cDNA, synthetic DNA or genomic DNA. The DNA may be double-stranded or single-stranded, and if single-stranded, can be either the coding strand or non-coding strand. An RNA analog may be, for example, mRNA or a combination of 20 ribo- and deoxyribonucleotides. Illustrative examples of a polynucleotide of the invention are shown in Figs. 3, 5, 7, 9, 11, 13 and 15.

A polynucleotide of the invention typically is at least 15 nucleotides (or base pairs, bp) in length. In 25 some embodiments, a polynucleotide is about 20 to 100 nucleotides in length, or about 100 to 500 nucleotides in length. In other embodiments, a polynucleotide is greater than about 1500 nucleotides in length and encodes a polypeptide having the amino acid sequence shown in 30 Figs. 4, 6, 8, 10, 12, 14 or 16.

In some embodiments, a polynucleotide of the invention encodes analogs or derivatives of a polypeptide having the deduced amino acid sequence of Figs. 4, 6, 8,

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10, 12, 14 or 16. Such fragments, analogs or derivatives include, for example, naturally occurring allelic variants, non-naturally occurring allelic variants, deletion variants and insertion variants, that do not 5 substantially alter the function of the polypeptide.

A polynucleotide of the invention may further comprise additional nucleic acids. For example, a nucleic acid fragment encoding a secretory or leading amino acid sequence can be fused in-frame to the amino 10 terminal end of one of the EL1 through EL7 polypeptides. Other nucleic acid fragments are known in the art that encode amino acid sequences useful for fusing in-frame to the KAS polypeptides disclosed herein. See, e.g., U.S. 5,629,193 incorporated herein by reference. A 15 polynucleotide may further comprise one or more regulatory elements operably linked to a KAS polynucleotide disclosed herein.

The present invention also comprises polynucleotides that hybridize to a KAS polynucleotide 20 disclosed herein. Such a polynucleotide typically is at least 15 nucleotides in length. Hybridization typically involves Southern analysis (Southern blotting), a method by which the presence of DNA sequences in a target nucleic acid mixture are identified by hybridization to a 25 labeled oligonucleotide or DNA fragment probe. Southern analysis typically involves electrophoretic separation of DNA digests on agarose gels, denaturation of the DNA after electrophoretic separation, and transfer of the DNA to nitrocellulose, nylon, or another suitable membrane 30 support for analysis with a radiolabeled, biotinylated, or enzyme-labeled probe as described in sections 9.37- 9.52 of Sambrook et al., (1989) *Molecular Cloning*, second edition, Cold Spring Harbor Laboratory, Plainview, NY.

A polynucleotide can hybridize under moderate 35 stringency conditions or, preferably, under high

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stringency conditions to a KAS polynucleotide disclosed herein. High stringency conditions are used to identify nucleic acids that have a high degree of homology to the probe. High stringency conditions can include the use of 5 low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (0.1X SSC); 0.1% sodium lauryl sulfate (SDS) at 65°C. Alternatively, a denaturing agent such as formamide can be employed during hybridization, e.g., 50% formamide with 0.1% 10 bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is the use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium 15 phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Moderate stringency conditions refers to

20 hybridization conditions used to identify nucleic acids that have a lower degree of identity to the probe than do nucleic acids identified under high stringency conditions. Moderate stringency conditions can include the use of higher ionic strength and/or lower 25 temperatures for washing of the hybridization membrane, compared to the ionic strength and temperatures used for high stringency hybridization. For example, a wash solution comprising 0.060 M NaCl/0.0060 M sodium citrate (4X SSC) and 0.1% sodium lauryl sulfate (SDS) can be used 30 at 50°C, with a last wash in 1X SSC, at 65°C. Alternatively, a hybridization wash in 1X SSC at 37°C can be used.

Hybridization can also be done by Northern analysis (Northern blotting), a method used to identify 35 RNAs that hybridize to a known probe such as an

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oligonucleotide, DNA fragment, cDNA or fragment thereof, or RNA fragment. The probe is labeled with a radioisotope such as ^{32}P , by biotinylation or with an enzyme. The RNA to be analyzed can be usually
5 electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook
10 et al., *supra*.

A polynucleotide has at least about 70% sequence identity, preferably at least about 80% sequence identity, more preferably at least about 90% sequence identity to SEQ ID NO:1, 3, 5, 7, 9, 11, or 13. Sequence
15 identity can be determined, for example, by computer programs designed to perform single and multiple sequence alignments.

A polynucleotide of the invention can be obtained by chemical synthesis, isolation and cloning from plant
20 genomic DNA or other means known to the art, including the use of PCR technology carried out using oligonucleotides corresponding to portions of SEQ ID NO:1, 3, 5, 7-9, 11 or 13. Polymerase chain reaction (PCR) refers to a procedure or technique in which target
25 nucleic acid is amplified in a manner similar to that described in U.S. Patent No. 4,683,195, incorporated herein by reference, and subsequent modifications of the procedure described therein. Generally, sequence information from the ends of the region of interest or
30 beyond is employed to design oligonucleotide primers that are identical or similar in sequence to opposite strands of the template to be amplified. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total
35 cellular RNA, bacteriophage or plasmid sequences, and the

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like. Alternately, a cDNA library (in an expression vector) can be screened with KAS-specific antibody prepared using peptide sequence(s) from hydrophilic regions of the KAS protein of SEQ ID NO:2 and technology known in the art.

A polypeptide of the invention comprises an isolated polypeptide having the deduced amino acid sequence of Figs. 2, 4, 6, 8, 10 and 12, as well as derivatives and analogs thereof. By "isolated" is meant a polypeptide that is expressed and produced in an environment other than the environment in which the polypeptide is naturally expressed and produced. For example, a plant polypeptide is isolated when expressed and produced in bacteria or fungi. Similarly, a plant polypeptide is isolated when its gene coding sequence is operably linked to a chimeric regulatory element and expressed in a tissue where the polypeptide is not naturally expressed. A polypeptide of the invention also comprises variants of the KAS polypeptides disclosed herein, as discussed above.

A full-length KAS coding sequence may comprise the sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13. Alternatively, a chimeric full-length KAS coding sequence may be formed by linking, in-frame, nucleotides from the 5' region of a first KAS gene to nucleotides from the 3' region of a second KAS gene, thereby forming a chimeric KAS protein.

It should be appreciated that nucleic acid fragments having a nucleotide sequence other than the KAS sequences disclosed in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 will encode a polypeptide having the exemplified amino acid coding sequence of SEQ ID NO:2, 4, 6, 8, 10, 12 or 14, respectively. The degeneracy of the genetic code is well-known to the art; i.e., for many amino acids, there

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is more than one nucleotide triplet which serves as the codon for the amino acid.

It should also be appreciated that certain amino acid substitutions can be made in protein sequences

5 without affecting the function of the protein.

Generally, conservative amino acid substitutions or substitutions of similar amino acids are tolerated without affecting protein function. Similar amino acids can be those that are similar in size and/or charge

10 properties, for example, aspartate and glutamate and isoleucine and valine are both pairs of similar amino acids. Similarity between amino acid pairs has been assessed in the art in a number of ways. For example, Dayhoff et al. (1978) in *Atlas of Protein Sequence and*

15 *Structure*, Vol. 5, Suppl. 3, pp. 345-352, which is incorporated by reference herein, provides frequency tables for amino acid substitutions which can be employed as a measure of amino acid similarity.

A nucleic acid construct of the invention

20 comprises a polynucleotide as disclosed herein linked to another, different polynucleotide. For example, a full-length KAS coding sequence may be operably fused in-frame to a nucleic acid fragment that encodes a leader sequence, secretory sequence or other additional amino

25 acid sequences that may be usefully linked to a polypeptide or peptide fragment.

A transgenic plant of the invention contains a nucleic acid construct as described herein. In some embodiments, a transgenic plant contains a nucleic acid 30 construct that comprises a polynucleotide of the invention operably linked to at least one suitable regulatory sequence in sense orientation. Regulatory sequences typically do not themselves code for a gene product. Instead, regulatory sequences affect the expression level of the polynucleotide to which they are

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linked. Examples of regulatory sequences are known in the art and include, without limitation, minimal promoters and promoters of genes preferentially or exclusively expressed in seeds or in epidermal cells of stems and leaves. Native regulatory sequences of the polynucleotides disclosed herein can be readily isolated by those skilled in the art and used in constructs of the invention. Other examples of suitable regulatory sequences include enhancers or enhancer-like elements, introns, 3' non-coding regions such as poly A sequences and other regulatory sequences discussed herein. Molecular biology techniques for preparing such chimeric genes are known in the art.

In other embodiments, a transgenic plant contains a nucleic acid construct comprising a partial or a full-length KAS coding sequence operably linked to at least one suitable regulatory sequence in antisense orientation. The chimeric gene can be introduced into a plant and transgenic progeny displaying expression of the antisense construct are identified.

One may use a polynucleotide disclosed herein for cosuppression as well as for antisense inhibition. Cosuppression of genes in plants may be achieved by expressing, in the sense orientation, the entire or partial coding sequence of a gene. See, e.g., WO 04\11516, incorporated herein by reference.

Transgenic techniques for use in the invention include, without limitation, *Agrobacterium*-mediated transformation, viral vector-mediated transformation electroporation and particle gun transformation.

Illustrative examples of transformation techniques are described in U.S. Patent 5,204,253, (particle gun) and U.S. Patent 5,188,958 (*Agrobacterium*), incorporated herein by reference. Transformation methods utilizing the Ti and Ri plasmids of *Agrobacterium spp.* typically

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use binary-type vectors. Walkerpeach, C. et al., in Plant Molecular Biology Manual, S. Gelvin and R. Schilperoort, eds., Kluwer Dordrecht, C1:1-19 (1994). If cell or tissue cultures are used as the recipient tissue 5 for transformation, plants can be regenerated from transformed cultures by techniques known to those skilled in the art.

Techniques are known for the introduction of DNA into monocots as well as dicots, as are the techniques 10 for culturing such plant tissues and regenerating those tissues. Monocots which have been successfully transformed and regenerated include wheat, corn, rye, rice, and asparagus. See, e.g., U.S. Patent Nos. 5,484,956 and 5,550,318, incorporated herein by 15 reference.

For efficient production of transgenic plants from plant cells, it is desirable that the plant tissue used for transformation possess a high capacity for regeneration. Transgenic plants of woody species such as 20 poplar and aspen have also been obtained. Technology is also available for the manipulation, transformation, and regeneration of gymnosperm plants. For example, U.S. Patent No. 5,122,466 describes the biotic transformation of conifers, with preferred target tissue 25 being meristematic and cotyledon and hypocotyl tissues. U.S. Patent No. 5,041,382 describes enrichment of conifer embryonal cells.

Seeds produced by a transgenic plant(s) can be grown and then selfed (or outcrossed and selfed) to 30 obtain seeds homozygous for the construct. Seeds can be analyzed in order to identify those homozygotes having the desired expression of the construct. Transgenic plants may be entered into a breeding program, e.g., to introgress the novel construct into other lines, to 35 transfer the construct to other species, or for further

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selection of other desirable traits. Alternatively, transgenic plants may be propagated vegetatively for those species amenable to such techniques. A nucleic acid construct of the invention can alter the levels of 5 very long chain fatty acids in plant tissues expressing the polynucleotide, compared to VLCFA levels in corresponding tissues from an otherwise identical plant not expressing the polynucleotide. A comparison can be made, for example, between a non-transgenic plant of a 10 plant line and a transgenic plant of the same plant line. Levels of VLCFAs having 20-32 carbons and/or levels of VLCFAs having 32-60 carbons can be altered in plants disclosed herein. Plants having an altered VLCFA composition may be identified by techniques known to the 15 skilled artisan, e.g., thin layer chromatography or gas-liquid chromatography (GLC) analysis of the appropriate plant tissue.

A suitable group of plants with which to practice the invention are the *Brassica* species, including *B. napus*, *B. rapa*, *B. juncea*, and *B. hirta*. Other suitable 20 plants include, without limitation, soybean (*Glycine max*), sunflower (*Helianthus annuus*) and corn (*Zea mays*).

A method according to the invention comprises introducing a nucleic acid construct into a plant cell 25 and producing a plant (as well as progeny of such a plant) from the transformed cell. Progeny includes descendants of a particular plant or plant line, e.g., seeds developed on an instant plant are descendants. Progeny of an instant plant include seeds formed on F_1 , 30 F_2 , F_3 , and subsequent generation plants, or seeds formed on BC_1 , BC_2 , BC_3 , and subsequent generation plants.

Methods and compositions according to the invention are useful in that the resulting plants and plant lines have desirable alterations in very long chain 35 fatty acid composition. Suitable tissues in which to

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express polynucleotides and/or polypeptides of the invention include, without limitation, seeds, stems and leaves. Leaf tissues of interest include cells and tissues of the epidermis, e.g., cells that are involved 5 in forming trichomes. Of particular interest are epidermal cells involved in forming the cuticular layer. The cuticular layer comprises various very long chain fatty acids and VLCFA derivatives such as alkanes, esters, alcohols and aldehydes. Altering the composition 10 and amount of VLCFAs in epidermal cells and tissues may enhance defense mechanisms and drought tolerance of plants disclosed herein.

Polynucleotides of the invention can be used as markers in plant genetic mapping and plant breeding 15 programs. Such markers may include RFLP, RAPD, or PCR markers, for example. Marker-assisted breeding techniques may be used to identify and follow a desired fatty acid composition during the breeding process. Marker-assisted breeding techniques may be used in 20 addition to, or as an alternative to, other sorts of identification techniques. An example of marker-assisted breeding is the use of PCR primers that specifically amplify a sequence from a desired KAS that has been introduced into a plant line and is being crossed into 25 other plant lines.

Plants and plant lines disclosed herein preferably have superior agronomic properties. Superior agronomic characteristics include, for example, increased seed germination percentage, increased seedling vigor, 30 increased resistance to seedling fungal diseases (damping off, root rot and the like), increased yield, and improved standability.

While the invention is susceptible to various modifications and alternative forms, certain specific 35 embodiments thereof are described in the general methods

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and examples set forth below. It should be understood, however, that these examples are not intended to limit the invention to the particular forms disclosed but, instead the invention is to cover all modifications, 5 equivalents and alternatives falling within the scope of the invention.

EXAMPLES

Example 1

Cloning and Expression of FAE1 in Yeast Cells

10 The open reading frame of the *Arabidopsis FAE1* gene was amplified directly by PCR, using *Arabidopsis thaliana* cv. Columbia genomic DNA as a template, pfu DNA polymerase and the following primers:
5' CTCGAGGAGCAATGACGTCCGTTAA-3' and 5'-
15 CTCGAGTTAGGACCGACCGTTTG-3'. The PCR product was blunt-end cloned into the Eco RV site of pBluescript (Stratagene, La Jolla, CA),

The *FAE1* gene was excised from the Bluescript vector with BamH1, and then subcloned into the pYEUra3 20 (Clontech, Palo Alto, CA). pYEUra3 is a yeast centromere-containing, episomal plasmid that is propagated stably through cell division. The *FAE1* gene was inserted downstream of a GAL1 promoter in pYEUra3. The GAL1 promoter is induced when galactose is present in 25 the medium and repressed when glucose is present in the growth medium.

Insertion of the *FAE1* gene in the sense orientation was confirmed by PCR, and pYEUra3/*FAE1* was used to transform *Saccharomyces cerevisiae* strain AB1380 30 using a lithium acetate procedure as described in Gietz, R. and Woods, R., in Molecular Genetics of Yeast: Practical Approaches, Oxford Press, pp. 121-134 (1994). Plasmid DNA was isolated from putative transformants, and

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the presence of the *FAE1/pYEUra3* construct was confirmed by Southern analysis.

Yeast transformed with pYEUra3 having *FAE1* operably linked to the *GAL1* promoter were grown in the presence of galactose or glucose and were analyzed for the expression of *FAE1*. As a control, yeast transformed with pYEUra3 containing no insert were also assayed. Analysis of such control preparations yielded fatty acid compositions and fatty acid elongation rates similar to those of yeast transformed with pYEUra3/*FAE1* and grown with glucose as the carbon source.

The fatty acid composition of yeast cells grown in the presence of galactose was compared to that of cells grown in the presence of glucose, to determine if VLCFA were found in the galactose-induced cells.

Transformed yeast cells were grown overnight in YPD media at 30°C with vigorous shaking. One hundred μ l of the overnight culture were used to inoculate 40 ml of complete minimal uracil dropout media (CM-Ura) supplemented with either glucose or galactose (2% w/v). Cultures were grown at 30°C to an OD₆₀₀ of approximately 1.3 to 1.5. Cells were harvested by centrifugation at 5000 Xg for 10 min. Total lipids were extracted from the cells with 2 volumes of 4N KOH in 100% methanol for 60 min. at 80°C. Fatty acids were saponified and methyl esters were prepared by drying the samples and resuspending in 0.5 ml of boron trichloride in methanol (10% v/v). Samples were incubated at 50°C for 15 min in a sealed tube. About 2 ml of water was then added and the fatty methyl esters were extracted thrice with 1 ml of hexane. Extracts were dried under nitrogen and redissolved in hexane. See Hlousek-Radojcic, A. et al., Plant J. 8:803-809. Methyl esters were analyzed on an HP 5890 series II gas chromatograph equipped with a 5771MSD and 7673 auto injector (Hewlett-Packard, Cincinnati, OH).

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Methyl esters were separated on a DB-23 (J&W Scientific) capillary column (30 m X 0.25 mm X 0.25 μm). The column was operated with helium carrier gas and splitless injection (injection temperature 280°C, detector 5 temperature 280°C). After an initial 3 min. at 100°C, the oven temperature was raised to 250° at 20°C min⁻¹ and maintained at that temperature for an additional 3 min. The identity of the peaks was verified by cochromatography with authentic standards and by mass 10 spectrometer analysis.

The results clearly revealed the appearance of both 20:1 and 22:1 acyl-CoA products in galactose-induced yeast containing the *FAE1* coding sequence. Uninduced yeast cells failed to accumulated significant amounts of 15 fatty acids longer than C18. These results indicate that expression of *FAE1* in yeast resulted in functional KAS activity and functional elongase activity.

Example 2

FAE1 Activity in Yeast Microsomes

20 The functional expression of the *FAE1* KAS was analyzed by isolating microsomes from transformed yeast cells and assaying these microsomes *in vitro* for elongase activity.

Transformed yeast cells were grown in the presence 25 of either glucose or galactose (2% w/v) as described in Example 1. Cells were harvested by centrifugation at 5000 Xg for 10 min and washed with 10 ml ice cold isolation buffer (IB), which contains 80 mM Hepes-KOH, pH 7.2, 5 mM EGTA, 5 mM EDTA, 10 mM KCl, 320 mM sucrose and 30 2 mM DTT). Cells were then resuspended in enough IB to fill a 1.7 ml tube containing 700 μl of 0.5 μm glass beads and yeast microsomes were isolated from the cells essentially as described in Tillman, T. and Bell, R., J. Biol. Chem. 261:9144-9149 (1986). The microsomal

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membrane pellet was recovered by centrifugation at 252,000 xg for 60 min. The pellet was rinsed by resuspending in 40 ml fresh IB and again recovered by centrifugation at 252000 Xg for 60 min. Microsomal 5 pellets were resuspended in a minimal volume of IB, and the protein concentration adjusted to 2.5 $\mu\text{g } \mu\text{l}^{-1}$ by addition of IB containing 15% glycerol. Microsomes were frozen on dry ice and stored at -80°C. The protein concentration in microsomes was determined by the 10 Bradford method (Bradford, 1976).

Fatty acid elongase activity was measured essentially as described in Hlousek-Radojcic, A. et al., Plant J. 8:803-809 (1995). Briefly, the standard elongation reaction mix contained 80 mM Hepes-KOH, pH 15 7.2, 20 mM MgCl₂, 500 μM NADPH, 1 mM ATP, 100 μM malonyl-CoA, 10 μM CoA-SH and 15 μM radioactive acyl-CoA substrate. The radiolabeled substrate was either [1-¹⁴C]18:1-CoA (50 uCi μmol^{-1}), [1-¹⁴C]18:0-CoA (55 uCi μmol^{-1}), or [1-¹⁴C]16:0-CoA (54 uCi μmol^{-1}). The reaction was 20 initiated by the addition of yeast microsomes (5 μg protein) and the mixture incubated at 30° C for the indicated period of time. The final reaction volume was 25 μl .

Methyl esters of the acyl-CoA elongation products 25 were prepared as described in Example 1. Methyl esters were separated on reversed phase silica gel KC18 TLC plates (Whatman, 250 μm thick), quantified by phosphorimaging, and analyzed on by ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA). The detection 30 limit for each product is about 0.001 nanomoles per min. per mg microsomal protein, depending on the phosphorimage exposure time.

Results of representative *in vitro* elongation assays are shown in Figs. 1 and 2. The results indicate 35 that microsomes from galactose-induced cells expressing

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FAE1 catalyzed multiple cycles of elongation starting with either C16:0 acyl CoA, C18:0 acyl CoA, or C18:1 acyl-CoA as the substrate (Fig. 1). The 16:0 and 18:0 acyl-CoA substrates were elongated to C26:0 acyl-CoA. In contrast, the 18:1-CoA substrate was elongated primarily to C20:1, with only low levels of C22:1 acyl-CoA being produced. Occasionally, trace levels of C24:1 CoA were also observed. Although the chain length of the products from the 18:1 acyl-CoA substrate were less than the chain length from the saturated acyl-CoA substrates, the rate of elongation of oleoyl-CoA was about 2- and 3-fold higher than the rates of elongation of 16:0-CoA and 18:0-CoA, respectively.

The elongation activity observed in microsomes from uninduced cells indicated that there was a low level of endogenous elongase activity when 18:1-CoA or 18:0-CoA were used as substrates. There was substantial 16:0-CoA elongase activity (10.1 nmol mg protein⁻¹ at 30 min) in microsomes from uninduced cells (Fig. 2). However, the major product of 16:0 elongation using uninduced microsomes was C18:0 acyl CoA, with only small amounts of products beyond this length. The elongation of the 16:0 acyl-CoA substrate presumably is due to an endogenous yeast elongase.

Elongation of 18:1 CoA by microsomes from induced cells occurred at a rate about 18-fold higher than in microsomes isolated from the uninduced cells (Fig. 2). With microsomes from induced yeast, synthesis of 20:0 CoA from the 16:0 CoA substrate, occurred at a rate similar to that seen when the substrate was 18:0 CoA (4.2 vs. 5.1 nmol mg protein⁻¹). The total rate of elongation of [¹⁴C] 16:0-CoA by microsomes from induced cells (15.8 nmol mg protein⁻¹ at 30 min.) was more than 50% higher than elongation of [¹⁴C] 16:0-CoA by microsomes from uninduced cells, suggesting that the FAE1 KAS utilized 16:0-CoA as

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a substrate in addition to C18-C24 acyl-CoAs. The *FAE1* elongase KAS activity, i.e., the difference in the 16:0 elongation rates between microsomes from induced and uninduced cells, was 5.7 nmol mg protein⁻¹. The 5 elongation rate with the 16:0 substrate thus was similar to the elongase activity of the *FAE1* elongase KAS with the 18:0 substrate.

These results indicate that *FAE1* KAS expressed in yeast could synthesize 3-ketoacyl-CoA *in vitro* and, in 10 combination with yeast reductases and dehydrases, could form a functional VLCFA elongase complex. In addition, these results suggest that *FAE1* is membrane-bound in yeast cells.

Example 3

15 **Cloning and Sequencing of *Arabidopsis* Elongase Genes**

The sequence of a jojoba seed cDNA (see WO 93/10241 and WO 95/15387, incorporated herein by reference) was used to search the *Arabidopsis* expressed sequence tag (EST) database of the *Arabidopsis* Genome 20 Stock Center (The Ohio State University, Columbus, Ohio). The BLAST computer program (National Institutes of Health, Bethesda, MD, USA) was used to perform the search. The search identified two ESTs (ATTS1282 and ATTS3218) that had a high degree of sequence identity 25 with the jojoba sequence. The ATTS1282 and ATTS3218 ESTs appeared to be partial cDNA clones rather than full-length clones based on the length of the jojoba sequence.

A genomic DNA library from *Arabidopsis thaliana* cv. Columbia, was prepared in the lambda GEM11 vector 30 (Promega, Madison, Wisconsin) and was obtained from Ron Davis, Stanford University, Stanford, CA. The library was hybridized with ATTS1282 and ATTS3218 as probes and 2 clones were identified for each EST. Phage DNA was isolated from each of the hybridizing clones, the genomic

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insert was excised with the restriction enzyme Sac I and subcloned into the plasmid pBluescript (Stratagene, La Jolla, CA). One clone from the ATTS1282 hybridization was designated EL1 and one clone from the ATTS3218 5 hybridization was designated EL2.

A yeast expression library, containing cDNA from *Arabidopsis thaliana* cv. Columbia, was prepared in the lambda YES expression vector described in Elledge et al. (Elledge, S. et al., Proc. Natl. Acad. Sci USA 88:1731-10 1735 (1991) and was obtained from Ron Davis at Stanford University, Stanford, CA. The library was hybridized with a EL2 partial cDNA probe. A full-length EL2 cDNA was not identified. However, the probe did identify a full-length cDNA which was designated EL3.

15 A consensus sequence for the C-terminal region of EL1, EL2 and the jojoba cDNA polypeptides was identified by sequence alignment using DNA analysis programs from DNASTar, Madison, Wisconsin. This consensus sequence was used to search the *Arabidopsis* EST database again for β -20 keto acyl synthase sequences. These searches identified four additional putative β -keto acyl synthase ESTs, which were designated EL4 through EL7. EL4, EL5, EL6, and EL7 have homology to Genbank Accession Nos. T04345, T44939, T22193 and T76700, respectively.

25 The lambda YES cDNA expression library described above was hybridized with the EL1 and EL4-EL7 ESTs as probes. This screen identified full-length cDNAs for EL1, EL5 and EL6.

The lambda GEM11 genomic library was hybridized 30 with the EL4 and EL7 ESTs as probes. This screen identified full-length genomic clones for EL4 and EL7. Phage DNA was isolated from each of the hybridizing clones and subcloned into pBluescript as described above.

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The 7 EL clones were sequenced on both strands with regions of overlap for each sequence run.

Sequencing was carried out with an ABI automated sequencer (Applied Biosystems, Inc., Foster City,

5 California), following the manufacturer's instructions.

The nucleotide sequences for the coding regions of EL1-EL7 are shown in Figs. 3, 5, 7, 9, 11, 13 and 15, respectively. The deduced amino acid sequences for EL1-

10 EL7 are shown in Figs. 4, 6, 8, 10, 12, 14 and 16, respectively, using the standard one-letter amino acid code. The EL1, EL2 and EL7 genomic clones appeared to lack introns. The EL4 genomic clone contained one intron near the 5' end of the coding region.

The nucleotide sequences of the 7 EL

15 polynucleotides were compared to 5 DNA sequences present in Genbank. Genbank, National Center for Biotechnology Information, Bethesda, MD. Two of the 5 accessions were cloned from members of the Brassicaceae: the *Arabidopsis* FAE1 sequence (Accession U29142) and a *Brassica napus* sequence (Accession U50771). Three of the accessions were cloned from jojoba (*Simmondsia chinensis*): 2 wax biosynthesis genes (Accessions I14084 and I14085) and a jojoba KAS gene (Accession U37088). See also U.S. Patent 5,445,947, incorporated herein by reference.

20 25 Multiple alignment of the 12 sequences was carried out with a computer program sold under the trade name MEGALIGN Lasergene by DNASTar (Madison, Wisconsin). Alignments were done using the Clustal method with weighted residue weight table. The nucleotide sequence 30 similarity index and percent divergence based on the multiple alignment algorithm is shown in Table 1. The nucleotide sequences of EL1-EL7 are distinguishable from the 5 DNA sequences obtained from Genbank.

The deduced amino acid sequences of the EL1-7 35 polypeptides were compared with the MEGALIGN program to

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the deduced amino acid sequences of the same 5 Genbank clones, using the Clustal method with PAM250 residue weight table. The amino acid sequence similarity and percent divergence are shown in Table 2. The amino acid 5 sequences of EL1-EL7 polypeptides are distinguishable from those of the Genbank sequences.

TABLE 1
Nucleotide sequence pair distances of EL1-EL7, using Clustal
method with weighted residue weight table.

	1	2	3	4	5	6	7	8	9	10	11	12	
1		77.5	62.4	58.8	57.0	54.9	47.0	42.8	42.9	43.1	44.7	41.3	ARAPAE1 U29142
2	18.1		61.0	57.9	55.4	53.7	46.9	42.7	44.1	42.9	42.3	40.5	BNAFAE1 U50771
3	40.4	41.0		70.5	59.3	56.4	46.7	48.5	48.1	48.6	46.5	43.5	EL2
4	43.9	44.3	28.0		56.3	55.4	46.5	47.0	45.1	47.2	47.4	42.3	EL3
5	40.7	42.3	45.0	45.0		68.0	54.0	46.8	46.6	46.4	49.0	47.2	EL5
6	45.8	48.9	46.0	47.3	32.4		53.6	48.6	48.2	48.6	49.0	49.2	EL7
7	74.1	71.0	69.4	67.3	64.3	64.5		49.8	49.2	49.8	47.7	48.2	EL6
8	68.1	66.2	63.4	63.1	65.5	64.2	56.1		97.7	99.7	48.4	45.8	JQJOKCS U37088
9	67.0	65.4	63.7	64.6	64.6	64.1	56.6	1.1		95.9	47.6	44.8	JOKCS10 I14084
10	67.2	65.2	61.8	61.4	64.1	63.0	56.3	0.2	1.1		48.4	45.3	JOKCS11 I14085
11	88.6	85.8	81.0	77.0	77.4	82.4	83.1	71.1	71.1	69.9		48.3	EL1
12	95.7	90.4	95.4	91.5	84.5	82.8	91.9	73.4	73.8	73.3	59.9		EL4
	1	2	3	4	5	6	7	8	9	10	11	12	

TABLE 2
 Amino acid sequence pair distances of EL1-EL7, using Clustal
 method with PAM250 residue weight table.

	1	2	3	4	5	6	7	8	9	10	11	12	
1	72.0	62.9	59.8	60.9	60.2	50.3	51.9	52.1	51.5	49.1	42.0	1	BL2
2	31.1	60.1	57.5	58.7	57.1	49.8	49.8	50.0	49.2	49.6	44.4	2	BL3
3	47.4	48.7	82.4	60.7	63.0	50.0	51.4	51.6	50.8	47.8	43.9	3	ATFAE1 U29142
4	51.8	52.8	17.9	60.2	61.0	49.2	50.3	50.5	49.7	46.5	42.4	4	BNFAE1 U50771
5	49.0	51.3	45.8	46.2	75.8	61.0	58.7	58.9	58.3	55.0	55.6	5	BL7
6	52.6	55.5	42.8	46.5	29.3	61.8	55.7	55.7	54.9	52.9	50.5	6	BL5
7	74.7	70.5	71.8	74.4	52.0	50.8	52.8	52.8	51.8	53.4	51.6	7	BL6
8	66.7	69.2	66.2	67.3	54.8	59.8	67.7	99.8	96.9	53.1	52.0	8	JOJCS U37088
9	66.3	68.7	66.2	67.3	54.0	58.3	67.7	0.2	96.9	53.1	51.9	9	JKCS11 I14085
10	66.3	69.7	66.6	67.8	54.5	60.7	68.6	1.6	51.7	50.7	10	JKCS10 I14084	
11	73.6	73.7	72.8	74.4	60.8	62.0	67.2	63.9	63.9	65.3	50.8	11	BL1
12	84.8	85.5	82.7	83.3	60.6	70.8	67.1	68.5	68.5	69.9	69.4	12	BL4
	1	2	3	4	5	6	7	8	9	10	11	12	

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Example 4

Expression of EL1 and EL2 in Yeast

The open reading frames (ORFs) for the EL2, EL4 and EL7 clones were amplified by PCR. The EL2 ORF was cloned into λYES using the primers:

CTCGAGCAAGTCCACTACCACGCA and CTCGAGCGAGTCAGAAGGAACAAA.

The EL4 ORF was cloned into pYEUra3 using the primers: GATAATTTAGAGAGGCACAGGGT and GTCGACACAAGAACGGTAGATCCAA.

The EL7 ORF was cloned into pYEUra3 using the primers: CAGTTCCCTAACGAAGCTA and GTCGACTTCTCAATGGACGGTGCCGGA. Amplified products were cloned into pYEUra3 under the control of, and 3' to, the GAL1 promoter. The resulting plasmids were transformed into yeast as described in Example 1.

Yeast cultures containing full-length EL1 in λYES and full-length EL2 in pYEUra3 were grown in the presence of galactose or glucose as described in Example 2. Microsomes were then prepared from each of the cultures and fatty acid elongation assays were carried out as described in Example 2.

In the first experiment, microsomes were prepared from galactose-induced cultures of EL1, EL2 and FAE1, and incubated with either [1-¹⁴C] 18:0 acyl-CoA or [1-¹⁴C] 18:1 acyl-CoA as substrate. The amounts of various reaction products synthesized after 30 minutes (min) were determined as described in Example 2. The results when 18:0 acyl-CoA was the substrate are shown in Table 3. The results when 18:1 acyl-CoA was the substrate are shown in Table 4.

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Table 3.
Elongation of 18:0-CoA by FAE1, EL1 and EL2 Genes
Expressed in Yeast

Acyl-CoA Product	β -Keto Acyl Synthase Gene					
	FAE1		EL1		EL2	
	Rate ¹	(%)	Rate	(%)	Rate	(%)
20:0	0.369	64.3	0.084	38.8	0.108	41.8
22:0	0.113	18.6	0.047	21.9	0.053	20.7
24:0	0.065	10.7	0.043	19.9	0.052	20.3
26:0	0.038	6.3	0.042	19.4	0.044	17.2
Total	0.585	100.0	0.216	100.0	0.258	100.0

¹ Nanomoles/minute/mg of microsomal protein

Table 4.
Elongation of 18:1-CoA by FAE1, EL1 and EL2 Genes
Expressed in Yeast

Acyl-CoA Product	β -Keto Acyl Synthase Gene					
	FAE1		EL1		EL2	
	Rate ¹	(%)	Rate	(%)	Rate	(%)
20:0	1.131	84.6	0.111	80.8	0.091	84.1
22:1	0.206	15.4	0.026	19.2	0.017	15.9
24:1	0.0	0.0	0.0	0.0	0.0	0.0
26:1	0.0	0.0	0.0	0.0	0.0	0.0
Total	1.337	100.0	0.137	100.0	0.108	100.0

¹ Nanomoles/minute/mg of microsomal protein

The results shown in Tables 3 and 4 indicate that the EL1 and EL2 gene products have β -ketoacyl synthase (KAS) activity and that the KAS reaction product can be utilized to form VLCFAs. The specific activities of the 3 KAS enzymes cannot be compared, since the relative amount of the heterologous KAS protein in each microsomal preparation is not known. However, the proportions of various reaction products can be compared between FAE1, EL1 and EL2.

The data shown in Table 3 indicate that the EL1 and EL2 KAS activities result in a higher proportion of saturated VLCFAs than does the FAE1 KAS activity. These

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results suggest that EL1 and EL2 encode novel gene products, because EL1 and EL2 have a greater preference for C22:0 and C24:0 acyl-CoA substrates than does FAE1.

A comparison of the relative elongation activity of FAE1 with 18:0 and 18:1 substrates (Tables 3 and 4) indicates that FAE1 is more active when 18:1 is the substrate than when 18:0 is the substrate. In contrast, the overall rate of product formation with EL1 is less when 18:1 is the substrate than when 18:0 is the substrate (Tables 3 and 4). EL2 is also less active when 18:1 is the substrate than when 18:0 is the substrate (Tables 3 and 4). These results support the conclusion that EL1 and EL2 encode novel gene products and suggest that EL1 and EL2 have a preference for saturated fatty acids as substrates, whereas the FAE1 gene product has a preference for monounsaturated fatty acids as substrates.

In a second experiment, microsomes were prepared from galactose-induced and from glucose-repressed yeast cultures containing EL1 or EL2 coding sequences. The microsomal preparations were incubated with either 18:0 acyl-CoA or 18:1 acyl-CoA as substrate and the fatty acid reaction products determined as described above. The results with the 18:0 substrate are shown in Table 5. The results with the 18:1 substrate are shown in Table 6.

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Table 5.
Elongation of 18:0-CoA by EL1 and EL2
With and Without Induction of Gene Expression

		β -Keto Acyl Synthase Gene							
Acyl CoA	EL1				EL2				
	+Glucose		+Galactose		+Glucose		+Galactose		
	Rate ¹	(%)	Rate	(%)	Rate	(%)	Rate	(%)	
20:0	0.007	100.0	0.074	55.8	0.030	81.3	0.107	43.1	
22:0	0.000	0.0	0.023	17.4	0.002	5.1	0.044	17.8	
24:0	0.000	0.0	0.020	15.3	0.005	13.6	0.048	19.1	
26:0	0.000	0.0	0.015	11.5	0.000	0.0	0.050	20.0	
Total	0.007	100.0	0.133	100.0	0.037	100.0	0.249	100.0	

Nanomoles/minute/mg of microsomal protein

Table 6.
Elongation of 18:1-CoA by EL1 and EL2
With and Without Induction of Gene Expression

		β -Keto Acyl Synthase Gene							
Acyl CoA	EL1				EL2				
	+Glucose		+Galactose		+Glucose		+Galactose		
	Rate ¹	(%)	Rate	(%)	Rate	(%)	Rate	(%)	
20:1	0.062	100.0	0.081	100.0	0.043	100.0	0.089	100.0	
22:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0	
24:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0	
26:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0	
Total	0.062	100.0	0.081	100.0	0.043	100.0	0.089	100.0	

Nanomoles/minute/mg of microsomal protein

The results in Table 5 show *in vitro* elongase activity for EL1 and EL2 under induced (galactose) and uninduced (glucose) conditions. The comparison indicates that induction with galactose results in a large increase in overall elongase activity when 18:0 acyl CoA is the substrate (about 19-fold and 7-fold for EL1 and EL2, respectively). In contrast, induction when 18:1 acyl CoA is the substrate results in only a small increase in elongase activity (about 1.3-fold and 2-fold for EL1 and EL2, respectively), as shown in Table 6.

The results in Table 5 show that little or no VLCFA products are made by yeast microsomes under uninduced conditions. Upon induction of EL1 and EL2 gene

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expression, however, significant quantities of C20:0, C22:0, C24:0 and C26:0 are made. The data in Tables 5 and 6 are consistent with the results in Tables 3 and 4, which indicated that EL1 and EL2 were more active with a saturated fatty acid substrate than with a monounsaturated substrate.

The data in Tables 5 and 6 are also consistent with the data in Tables 3 and 4 indicating that the EL1 and EL2 gene products are more active in converting C24:0 to C26:0 than is FAE1.

In a third experiment, microsomes from induced and uninduced cultures containing EL1 or EL2 were incubated in the absence of cofactors involved in the β -ketoacyl condensation reaction. Cultures were induced and microsomes were prepared as described in Example 2. *In vitro* assays were carried out as described in Example 2, except that either ATP, CoASH or both were omitted from the enzyme reaction mixture. In addition, one reaction was carried out in a complete mixture having 0.01 mM of cerulenin (Sigma, St. Louis, MO). Cerulenin is an inhibitor of some condensing enzymes. The results are shown in Tables 7-9.

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Table 7.
Effect of Cofactors on 18:0-CoA Elongation¹

Gene	Expt ⁴	+Glu ²	+Gal ²	-ATP ³	-CoA ³	-A&C ³	+ Cer ³
EL1	1	.037	.109	.095	.105	.119	.141
	2	N.D.	.090	.125	.093	.270	.176
EL2	1	.033	.112	.168	.127	.143	.238
	2	N.D.	.120	.178	.133	.195	.302

¹ Activity in nanomoles/minute/mg of microsomal protein.

² +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.

³ Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

⁴ Experiment No.

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Table 8.
Effect of Cofactors on Elongation Products of EL1¹

Prod.	+Glu ²	+Gal ²	-ATP ³	-CoA ³	-A&C ³	+Cer ³
20:0	53.9	46.2	34.4	47.8	41.7	46.7
22:0	14.4	18.7	13.7	18.0	19.4	16.2
24:0	18.5	18.1	20.6	19.1	16.7	17.7
26:0	13.2	17.1	31.4	15.2	22.3	19.4
Total	100.0	100.0	100.0	100.0	100.0	100.0

¹ Amount of indicated product as a percent of total products formed.
 Results of one experiment for +Glucose; Average of two experiments for other conditions.

² +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.

³ Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

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Table 9.
Effect of Cofactors on Elongation Products of EL2¹

Prod.	+Glu ²	+Gal ²	-ATP ³	-CoA ³	-A&C ³	+Cer ³
20:0	54.5	47.1	34.1	45.3	38.0	41.8
22:0	17.1	19.1	16.4	19.2	15.9	16.1
24:0	5.8	19.4	20.8	19.9	18.4	20.4
26:0	22.6	14.5	28.9	15.8	27.8	21.8
Total	100.0	100.0	100.0	100.0	100.0	100.0

¹ Amount of indicated product as a percent of total products formed.
 Results of one experiment for +Glucose; Average of two experiments for other conditions.

² +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.

³ Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

The results in Table 7 indicate that omission of ATP and/or CoA from the incubation mixture does not have a significant effect on the overall amounts of VLCFAs synthesized by the *in vitro* KAS activity of EL1 or EL2. The results also show that cerulenin does not inhibit the KAS activity of EL1 or EL2. The data in Table 8 and 9 confirm that EL1 and EL2 KAS activity produces significant amounts of C24:0 and C26:0 acyl CoA products.

To the extent not already indicated, it will be understood by those of ordinary skill in the art that any one of the various specific embodiments herein described and illustrated may be further modified to incorporate features shown in other of the specific embodiments.

The foregoing detailed description has been provided for a better understanding of the invention only and no unnecessary limitation should be understood therefrom as some modifications will be apparent to those

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skilled in the art without deviating from the spirit and scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: CARGILL, INCORPORATED

(ii) TITLE OF THE INVENTION: FATTY ACID ELONGASES

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Fish & Richardson P.C., P.A.
- (B) STREET: 60 South Sixth Street, Suite 3300
- (C) CITY: Minneapolis
- (D) STATE: MN
- (E) COUNTRY: USA
- (F) ZIP: 55402

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Diskette
- (B) COMPUTER: IBM Compatible
- (C) OPERATING SYSTEM: DOS
- (D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: 08/868,373
- (B) FILING DATE: 03-JUN-1997

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Lundquist, Ronald C
- (B) REGISTRATION NUMBER: 37,875
- (C) REFERENCE/DOCKET NUMBER: 07039/064W01

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 612-335-5050
- (B) TELEFAX: 612-288-9696
- (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1560 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGATCGAG AGAGATTAAC GGCGGAGATG GCGTTTCGAG ATTCAATCATC GGCCGTTATA	60
AGAATTGAA GACGTTTGCC GGATTTATTA ACGTCCGTTA AGCTCAAATA CGTGAAGCTT	120
GGACTTCACA ACTCTTGAA CGTGACCACC ATTCTCTTCT TCTTAATTAT TCTTCCTTTA	180
ACCGGAACCG TGCTGGTICA GCTAACCGGGT CTAACGTTCG ATACGTTCTC TGAGCTTTGG	240
TCTAACCGAG CGGTCAACT CGACACGGCG ACGAGACTTA CCTGCTTGGT TTTCCCTCTCC	300
TTCGTTTGA CCCTCTACGT GGCTAACCGG TCTAAACCGG TTTACCTAGT GGATTTCTCC	360
TGCTACAAAC CGGAAGACGA CGCTAAAATA TCAGTAGATT CGTTCTTGAC GATGACTGAG	420
GAAAATGGAT CATTCAACCGA TGACACGGTT CAGTTCCAGC AAAGAATCTC GAACCGGGCC	480

GGTTTGGGAG	ACGAGACGTA	TCTGCCACGT	GGCATAACTT	CAACGCCCCC	GAAGCTAAAT	540
ATGTCAGAGG	CACGTGCCGA	AGCTGAAGCC	GTATGTTTG	GAGCCTTAGA	TTCCCTCTTC	600
GAGAAAAACCG	GAATTAAACC	GGCCGAAGTC	GGAATCTTG	TAGTAAACTG	CAGCTTATTTC	660
AATCCGACGC	CGTCTCTATC	AGCGATGATC	GTGAACCAT	ACAAGATGAG	AGAAGACATC	720
AAAAGTTACA	ACCTCGGAGG	AATGGGTTGC	TCCGCCGGAT	TAATCTCAAT	CGATCTCGCT	780
AACAATCTCC	TCAAAGCAAA	CCCTAATTCT	TACGCTGTCG	TGGTAAGCAC	GGAAAACATA	840
ACCTAAACT	GGTACTTCGG	AAATGACCGG	TCAATGCTCC	TCTGCAACTG	CATCTCCGA	900
ATGGGCGGAG	CTCGGATTCT	CCTCTCTAAC	CGCCGTCAG	ACCGGAAGAA	GTCAAAGTAC	960
TCGCTGGTCA	ACGTGTTCG	AACACATAAA	GGATCAGACG	ACAAGAACTA	CAATTGCGTG	1020
TACCAGAAGG	AAGACGAGAG	AGGAACAATC	GGTGTCTCTT	TAGCTAGAGA	GCTCATGTCT	1080
GTCCGCCGGAG	ACGCTCTGAA	AACAAACATC	ACGACTTTAG	GACCGATGGT	TCTTCCATTG	1140
TCAGAGCAGT	TGATGTTCTT	GATTTCCCTG	GTCAAAAGGA	AGATGTTCAA	GTAAAAGTT	1200
AAACCGTATA	TTCCGGATT	CAAGCTAGCT	TTCGACCTT	TCTGTATTCA	CGCAGGAGGT	1260
AGAGCGGTT	TAGACGAAGT	CGAGAAGAA	CTTGATCTCA	AAGATTGGCA	CATGGAACCT	1320
TCTAGAATGA	CTTGCACAG	ATTTGGTAAC	ACTCGAGTA	GCTCGCTTG	GTATGAGATG	1380
GCTTATACCG	AAGCTAAGGG	TCGGGTTAAA	GCTGGTGACC	GACTTTGGCA	GATTGCGTT	1440
GGATCGGGTT	TCAAGTGTAA	TAGTGCCTT	TGGAAAGCGT	TACGACCGGT	TTCGACGGAG	1500
GAGATGACCG	GTAATGCTTG	GGCTGGTTCG	ATTGATCAAT	ATCCGGTTAA	AGTTGTGCAA	1560

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 520 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asp	Arg	Glu	Arg	Leu	Thr	Ala	Glu	Met	Ala	Phe	Arg	Asp	Ser	Ser
1					5				10					15	
Ser	Ala	Val	Ile	Arg	Ile	Arg	Arg	Arg	Leu	Pro	Asp	Leu	Leu	Thr	Ser
									20			25		30	
Val	Lys	Leu	Lys	Tyr	Val	Lys	Leu	Gly	Leu	His	Asn	Ser	Cys	Asn	Val
									35			40		45	
Thr	Thr	Ile	Leu	Phe	Phe	Leu	Ile	Ile	Leu	Pro	Leu	Thr	Gly	Thr	Val
									50			55		60	
Leu	Val	Gln	Leu	Thr	Gly	Leu	Thr	Phe	Asp	Thr	Phe	Ser	Glu	Leu	Trp
									65			70		75	
Ser	Asn	Gln	Ala	Val	Gln	Leu	Asp	Thr	Ala	Thr	Arg	Leu	Thr	Cys	Leu
									85			90		95	
Val	Phe	Leu	Ser	Phe	Val	Leu	Thr	Leu	Tyr	Val	Ala	Asn	Arg	Ser	Lys
									100			105		110	
Pro	Val	Tyr	Leu	Val	Asp	Phe	Ser	Cys	Tyr	Lys	Pro	Glu	Asp	Glu	Arg
									115			120		125	
Lys	Ile	Ser	Val	Asp	Ser	Phe	Leu	Thr	Met	Thr	Glu	Glu	Asn	Gly	Ser
									130			135		140	
Phe	Thr	Asp	Asp	Thr	Val	Gln	Phe	Gln	Gln	Arg	Ile	Ser	Asn	Arg	Ala
									145			150		155	
Gly	Leu	Gly	Asp	Glu	Thr	Tyr	Leu	Pro	Arg	Gly	Ile	Thr	Ser	Thr	Pro
									165			170		175	
Pro	Lys	Leu	Asn	Met	Ser	Glu	Ala	Arg	Ala	Glu	Ala	Glu	Ala	Val	Met
									180			185		190	
Phe	Gly	Ala	Leu	Asp	Ser	Leu	Phe	Glu	Lys	Thr	Gly	Ile	Lys	Pro	Ala
									195			200		205	
Glu	Val	Gly	Ile	Leu	Ile	Val	Asn	Cys	Ser	Leu	Phe	Asn	Pro	Thr	Pro
									210			215		220	
Ser	Leu	Ser	Ala	Met	Ile	Val	Asn	His	Tyr	Lys	Met	Arg	Glu	Asp	Ile
									225			230		235	
Lys	Ser	Tyr	Asn	Leu	Gly	Gly	Met	Gly	Cys	Ser	Ala	Gly	Leu	Ile	Ser
									245			250		255	
Ile	Asp	Leu	Ala	Asn	Asn	Leu	Leu	Lys	Ala	Asn	Pro	Asn	Ser	Tyr	Ala
									260			265		270	
Val	Val	Val	Ser	Thr	Glu	Asn	Ile	Thr	Leu	Asn	Trp	Tyr	Phe	Gly	Asn
									275			280		285	

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Asp	Arg	Ser	Met	Leu	Leu	Cys	Asn	Cys	Ile	Phe	Arg	Met	Gly	Gly	Ala
290						295						300			
Ala	Ile	Leu	Leu	Ser	Asn	Arg	Arg	Gln	Asp	Arg	Lys	Lys	Ser	Lys	Tyr
305						310					315				320
Ser	Leu	Val	Asn	Val	Val	Arg	Thr	His	Lys	Gly	Ser	Asp	Asp	Lys	Asn
						325				330				335	
Tyr	Asn	Cys	Val	Tyr	Gln	Lys	Glu	Asp	Glu	Arg	Gly	Thr	Ile	Gly	Val
						340				345				350	
Ser	Leu	Ala	Arg	Glu	Leu	Met	Ser	Val	Ala	Gly	Asp	Ala	Leu	Lys	Thr
						355				360				365	
Asn	Ile	Thr	Thr	Leu	Gly	Pro	Met	Val	Leu	Pro	Leu	Ser	Glu	Gln	Leu
						370				375				380	
Met	Phe	Leu	Ile	Ser	Leu	Val	Lys	Arg	Lys	Met	Phe	Lys	Leu	Lys	Val
						385				390				395	
Lys	Pro	Tyr	Ile	Pro	Asp	Phe	Lys	Leu	Ala	Phe	Glu	His	Phe	Cys	Ile
						405				410				415	
His	Ala	Gly	Gly	Arg	Ala	Val	Leu	Asp	Glu	Val	Gln	Lys	Asn	Leu	Asp
						420				425				430	
Leu	Lys	Asp	Trp	His	Met	Glu	Pro	Ser	Arg	Met	Thr	Leu	His	Arg	Phe
						435				440				445	
Gly	Asn	Thr	Ser	Ser	Ser	Leu	Trp	Tyr	Glu	Met	Ala	Tyr	Thr	Glu	
						450				455				460	
Ala	Lys	Gly	Arg	Val	Lys	Ala	Gly	Asp	Arg	Leu	Trp	Gln	Ile	Ala	Phe
						465				470				475	
Gly	Ser	Gly	Phe	Lys	Cys	Asn	Ser	Ala	Val	Trp	Lys	Ala	Leu	Arg	Pro
						485				490				495	
Val	Ser	Thr	Glu	Glu	Met	Thr	Gly	Asn	Ala	Trp	Ala	Gly	Ser	Ile	Asp
						500				505				510	
Gln	Tyr	Pro	Val	Lys	Val	Val	Gln								
						515				520					

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1479 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGATTACC	CCATGAAGAA	GGTAAAAATC	TTTTCAACT	ACCTCATGGC	GCATCGCTTC	60
AAGCTCTGCT	TCTTACCAATT	AATGGTTGCT	ATAGCGTGG	AGGCGTCTCG	TCTTTCCACA	120
CAAGATCTCC	AAAACCTTTTA	CCTCTACTTA	CAAAACAACC	ACACATCTCT	AACCATGTTC	180
TTCCCTTACCC	TCGCTCTCGG	GTCGACTCTT	TACCTCATGA	CCCGGCCCAA	ACCCGTTTAT	240
CTCGTTGACT	TTAGCTGCTA	CCTCCCACCG	TCGCATCTCA	AAGCCAGCAC	CCAGAGGATC	300
ATGCAACACG	TAAGGCTTGT	ACGAGAACGA	GGCGCTGGA	AGCAAGAGTC	CGATTACTTG	360
ATGGACTCT	GCGAGAACAT	TCTAGAACGT	TCCCGTCTAG	GCCAAGAGAC	GTACGTACCC	420
GAAGGCTTC	AAACCTTGCC	ACTACAACAG	AATTGCGCT	TATCACGTAT	AGAGACGGAG	480
GAAGTTATT	TTGGTGCCTG	CGATAATCTG	TTTCGAAACA	CGGGAATAAG	CCCTAGTGTAT	540
ATAGGTATAT	TTGGGGTGA	TTCAAGCACT	TTTAATCCAA	CACCTTCGCT	ATCAAGTATC	600
TTAGTGAATA	AGTTTAAACT	TAGGGATAAT	ATAAAAGAGCT	TGAATCTTG	TGGGATGGGG	660
TGTAGCGCTG	GAGTCATCGC	TATCGATGCG	GCTAAGAGCT	TGTTACAAGT	TCATAGAAC	720
ACTTATGCTC	TTGGTGGTAG	CACCGAGAAC	ATCACTCAAA	ACTTGTACAT	GGGTAACAAC	780
AAATCAATGT	TGGTTACAAA	CTGTTTGTG	CGTATAGGTG	GGGCGCGAT	TTTGCTTTCT	840
AACCGGTCTA	TAGATCGTAA	ACCGCAAAA	TACGAGCTTG	TTCAACCCGT	GCGGGTCCAT	900
ACCGGAGCAG	ATGACCGATC	CTATGAATGT	GCAACTCAAG	AAGAGGATGA	AGATGGCATA	960
GTTGGGTTT	CCTTGTCAAA	GAATCTACCA	ATGGTAGCTG	CAAGAACCT	AAAGATCAAT	1020
ATCGCAACTT	TGGGTCCGCT	TGTTCTTCCC	ATAAGCGAGA	AGTTTCACTT	CTTTGTGAGG	1080
TTCGTTAAAA	AGAAGTTTCT	CAACCCCAAG	CTAAAGCATT	ACATTCCGGA	TTTCAAGCTC	1140
GCATTCCGAGC	ATTCTGTAT	CCATGCGGGT	GGTAGAGCGC	TAATTGATGA	GATGGAGAAG	1200
AATCTTCATC	TAACCTCACT	AGACGTTGAG	GCTTCAAGAA	TGACATTACA	CAGGTTTGGT	1260
AATACCTCTT	CGAGCTCCAT	TTGGTACGAG	TTGGCTTACA	CAGAAGCCAA	AGGAAGGATG	1320
ACGAAAGGAG	ATAGGATTTC	GCAGATTGCG	TTGGGGTCAG	GTTTTAAGTG	TAATAGTTCA	1380

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GTTTGGGTGG CTCTTCGTAAC CGTCAAGCCT TCTACTAATA ATCCTTGGGA ACAGTGTCTA 1440
CACAAATATC CAGTTGAGAT CGATATAGAT TTAAAAGAG 1479

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 493 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Tyr Pro Met Lys Lys Val Lys Ile Phe Phe Asn Tyr Leu Met
 1 5 10 15
 Ala His Arg Phe Lys Leu Cys Phe Leu Pro Leu Met Val Ala Ile Ala
 20 25 30
 Val Glu Ala Ser Arg Leu Ser Thr Gln Asp Leu Gln Asn Phe Tyr Leu
 35 40 45
 Tyr Leu Gln Asn Asn His Thr Ser Leu Thr Met Phe Phe Leu Tyr Leu
 50 55 60
 Ala Leu Gly Ser Thr Leu Tyr Leu Met Thr Arg Pro Lys Pro Val Tyr
 65 70 75 80
 Leu Val Asp Phe Ser Cys Tyr Leu Pro Pro Ser His Leu Lys Ala Ser
 85 90 95
 Thr Gln Arg Ile Met Gln His Val Arg Leu Val Arg Glu Ala Gly Ala
 100 105 110
 Trp Lys Gln Glu Ser Asp Tyr Leu Met Asp Phe Cys Glu Lys Ile Leu
 115 120 125
 Glu Arg Ser Gly Leu Gly Gln Glu Thr Tyr Val Pro Glu Gly Leu Gln
 130 135 140
 Thr Leu Pro Leu Gln Gln Asn Leu Ala Val Ser Arg Ile Glu Thr Glu
 145 150 155 160
 Glu Val Ile Ile Gly Ala Val Asp Asn Leu Phe Arg Asn Thr Gly Ile
 165 170 175
 Ser Pro Ser Asp Ile Gly Ile Leu Val Val Asn Ser Ser Thr Phe Asn
 180 185 190
 Pro Thr Pro Ser Leu Ser Ser Ile Leu Val Asn Lys Phe Lys Leu Arg
 195 200 205
 Asp Asn Ile Lys Ser Leu Asn Leu Gly Gly Met Gly Cys Ser Ala Gly
 210 215 220
 Val Ile Ala Ile Asp Ala Ala Lys Ser Leu Leu Gln Val His Arg Asn
 225 230 235 240
 Thr Tyr Ala Leu Val Val Ser Thr Glu Asn Ile Thr Gln Asn Leu Tyr
 245 250 255
 Met Gly Asn Asn Lys Ser Met Leu Val Thr Asn Cys Leu Phe Arg Ile
 260 265 270
 Gly Gly Ala Ala Ile Leu Leu Ser Asn Arg Ser Ile Asp Arg Lys Arg
 275 280 285
 Ala Lys Tyr Glu Leu Val His Thr Val Arg Val His Thr Gly Ala Asp
 290 295 300
 Asp Arg Ser Tyr Glu Cys Ala Thr Gln Glu Glu Asp Glu Asp Gly Ile
 305 310 315 320
 Val Gly Val Ser Leu Ser Lys Asn Leu Pro Met Val Ala Ala Arg Thr
 325 330 335
 Leu Lys Ile Asn Ile Ala Thr Leu Gly Pro Leu Val Leu Pro Ile Ser
 340 345 350
 Glu Lys Phe His Phe Phe Val Arg Phe Val Lys Lys Lys Phe Leu Asn
 355 360 365
 Pro Lys Leu Lys His Tyr Ile Pro Asp Phe Lys Leu Ala Phe Glu His
 370 375 380
 Phe Cys Ile His Ala Gly Gly Arg Ala Leu Ile Asp Glu Met Glu Lys
 385 390 395 400
 Asn Leu His Leu Thr Pro Leu Asp Val Glu Ala Ser Arg Met Thr Leu
 405 410 415

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1512 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTACGTCAGG	GTAGAACAAA	GAAGTAAACAC	TTAAGCAAAA	CAATTGGTCC	TACTCTTAGG	60
TTATCTCCAA	TGAAGAACTT	AAAGATGGTT	TTCTTCAAGA	TCCTCTTAT	CTCTTTAATG	120
GCAGGATTAG	CCATGAAAGG	ATCTAAAGTC	AACGTAGAG	ATCTCAAAA	GTTCTCCCTC	180
CACCATACAG	AGAACAAACCT	CCAAACCCATA	AGCCCTTCTAT	TGTTCTTGT	CGTTTTGTG	240
TGGATCTCT	ACATGTTAAC	CGCACCTAAA	CCCGTTTAC	TTGTTGATTT	CTCTCTGCTAC	300
CTTCCACCGT	CGCATCTCAA	GGTCAGTATC	CAAAACCTAA	TGGGACACGC	AAGACGTGCA	360
AGAGAACAG	GCATGTTGTTG	GAAGAACAAA	GAGAGCGACC	ATTAGTTGA	CTTCCAGGAG	420
AAGATTCTTG	AACGTTCCGG	TCTTGGTCAA	GAAACCTACA	TCCCCGAGGG	TCTTCAGTGC	480
TTCCCACCTC	AGCAAGGCAT	GGGTGCTTCA	CGTAAAGAGA	CGGAAAGAAGT	AATCTTCGGA	540
GCTCTTGACA	ATCTTTTTCG	CAACACCGGT	GTAAAACCTG	ATGATATCGG	TATATTGGTG	600
GTGAAATTCA	GCACGTTAA	TCCAACCTCA	TCACTCGCC	CCATGATTGT	GAACAAAGTAC	660
AAACTCAGAG	ACAACATCAA	GAGTTTGAAT	CTTGGAGGG	TGGGGTCAG	TGCCGGAGGT	720
ATAGCTGTG	ATGTCGCTAA	GGGATTACTA	CAAGTTCAT	GGAACACTTA	TGCTTATTGTA	780
GTAAGCACAG	AGAACATCAC	TCAGAACCTTA	TACTTGGGG	AAAACAAATC	ATGCTAGTC	840
ACAAACTGTG	TGTTCCCGGT	TGGTGGTGC	CGGGTTCTGC	TTTCAAACAG	ATCTAGAGAC	900
CGTAACCCCG	CCAAATACGA	GCTTGTTCAC	ACCGTACCGA	TCCATACCGG	ATCAGATGAT	960
AGGTCTTCG	AATGTCGAC	ACAAAGAGAG	GATGAAGATG	GTATAATTGG	AGTTACCTTG	1020
ACAAAGAACAT	TACCTATTGGT	GGCTGCAAGG	ACTCTTAAGA	TAATATTCG	ACTTTGGGT	1080
CCTCTTGTAC	TTCCATTAAA	AGAGAACGTA	GCCTTCTTA	TACTTTTGT	CAAGAACAG	1140
TATTTCAAGC	CAGAGTTAAG	GAATTATACA	CCAGATTCA	AGCTGCC	TGAGCATTTC	1200
TGTATCCACG	CTGGTGGAG	AGCTCTAATA	GATGAGCTGG	AGAAGAACCT	TAAGCTTTCT	1260
CCGGTTACACG	TAGAGCCGTC	AAAATGACAA	CTACACAGGT	TTGGTAACAC	TTCTTCTAGC	1320
TCAAATCTGGT	ACGAGTTAGC	TTATACAGAA	GCTAAAGGAA	GGATGAAGGA	AGGAGATAGG	1380
ATTTGGCAGA	TTGCTTGGG	GTCAAGTTT	AACTGTAACA	GTTCAGTATG	GTTGGCTCTG	1440
CGAGACGTAA	AGCCTTCAGC	TAACAGTCCA	TGGGAAGACT	GTATGGATAG	ATATCCGGTT	1500
GAGATTGATA	TT					1512

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 504 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Leu Arg Gln Gly Arg Thr Lys Ser Lys His Leu Ser Lys Thr Ile Cys
   1           5           10          15
Pro Thr Leu Arg Leu Ser Pro Met Lys Asn Leu Lys Met Val Phe Phe
   20          25          30

```

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Lys Ile Leu Phe Ile Ser Leu Met Ala Gly Leu Ala Met Lys Gly Ser
 35 40 45
 Lys Ile Asn Val Glu Asp Leu Gln Lys Phe Ser Leu His His Thr Gln
 50 55 60
 Asn Asn Leu Gln Thr Ile Ser Leu Leu Leu Phe Leu Val Val Phe Val
 65 70 75 80
 Trp Ile Leu Tyr Met Leu Thr Arg Pro Lys Pro Val Tyr Leu Val Asp
 85 90 95
 Phe Ser Cys Tyr Leu Pro Pro Ser His Leu Lys Val Ser Ile Gln Thr
 100 105 110
 Leu Met Gly His Ala Arg Arg Ala Arg Glu Ala Gly Met Cys Trp Lys
 115 120 125
 Asn Lys Glu Ser Asp His Leu Val Asp Phe Gln Glu Lys Ile Leu Glu
 130 135 140
 Arg Ser Gly Leu Gly Glu Thr Tyr Ile Pro Glu Gly Leu Gln Cys
 145 150 155 160
 Phe Pro Leu Gln Gln Gly Met Gly Ala Ser Arg Lys Glu Thr Glu Glu
 165 170 175
 Val Ile Phe Gly Ala Leu Asp Asn Leu Phe Arg Asn Thr Gly Val Lys
 180 185 190
 Pro Asp Asp Ile Gly Ile Leu Val Val Asn Ser Ser Thr Phe Asn Pro
 195 200 205
 Thr Pro Ser Leu Ala Ser Met Ile Val Asn Lys Tyr Lys Leu Arg Asp
 210 215 220
 Asn Ile Lys Ser Leu Asn Leu Gly Gly Met Gly Cys Ser Ala Gly Val
 225 230 235 240
 Ile Ala Val Asp Val Ala Lys Gly Leu Leu Gln Val His Arg Asn Thr
 245 250 255
 Tyr Ala Ile Val Val Ser Thr Glu Asn Ile Thr Gln Asn Leu Tyr Leu
 260 265 270
 Gly Lys Asn Lys Ser Met Leu Val Thr Asn Cys Leu Phe Arg Val Gly
 275 280 285
 Gly Ala Ala Val Leu Leu Ser Asn Arg Ser Arg Asp Arg Asn Arg Ala
 290 295 300
 Lys Tyr Glu Leu Val His Thr Val Arg Ile His Thr Gly Ser Asp Asp
 305 310 315 320
 Arg Ser Phe Glu Cys Ala Thr Gln Glu Glu Asp Glu Asp Gly Ile Ile
 325 330 335
 Gly Val Thr Leu Thr Lys Asn Leu Pro Met Val Ala Ala Arg Thr Leu
 340 345 350
 Lys Ile Asn Ile Ala Thr Leu Gly Pro Leu Val Leu Pro Leu Lys Glu
 355 360 365
 Lys Leu Ala Phe Phe Ile Thr Phe Val Lys Lys Lys Tyr Phe Lys Pro
 370 375 380
 Glu Leu Arg Asn Tyr Thr Pro Asp Phe Lys Leu Ala Phe Glu His Phe
 385 390 395 400
 Cys Ile His Ala Gly Gly Arg Ala Leu Ile Asp Glu Leu Glu Lys Asn
 405 410 415
 Leu Lys Leu Ser Pro Leu His Val Glu Ala Ser Arg Met Thr Leu His
 420 425 430
 Arg Phe Gly Asn Thr Ser Ser Ser Ile Trp Tyr Glu Leu Ala Tyr
 435 440 445
 Thr Glu Ala Lys Gly Arg Met Lys Glu Gly Asp Arg Ile Trp Gln Ile
 450 455 460
 Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ser Val Trp Val Ala Leu
 465 470 475 480
 Arg Asp Val Lys Pro Ser Ala Asn Ser Pro Trp Glu Asp Cys Met Asp
 485 490 495
 Arg Tyr Pro Val Glu Ile Asp Ile
 500

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1650 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGGGTAGAT	CCAACGAGCA	AGATCTGCTC	TCTACCGAGA	TCGTTAACCG	TGGGATCGAA	60
CCATCCGGTC	CTAACGCCGG	CTCACCAACG	TTCTCGGTTA	GGGTCAAGGAG	ACGTTTGCT	120
GATTTTCTTC	AGTCGGTGAA	CTTGAAGTGT	GTGAAATTG	GTTACCACTA	CCTCATAAAC	180
CATGCGGTTT	ATTTCGGCAG	CATACCGGTT	CTTGTGCTGG	TTTTAGTGC	TGAGGTTGGG	240
AGTTTAAGCA	GAGAAGAGAT	TTGGAAGAAG	CTTGGGACT	ATGATCTTGC	AACGTGTTATC	300
GGATTCTTCG	GTGCTTTGT	TTAACCGCT	TGTGTCTACT	TCATGTCTCG	TCCTCGCTCT	360
GTTTATCTTA	TTGATTTTCGC	TTGTTACAAG	CCCTCCGATG	AACACAAGGT	GACAAAAGAA	420
GAGTTCATAG	AACTAGCAGG	AAAATCAGGG	AAGTTGACG	AAGAGACACT	CGGTTTCAAG	480
AAGAGGACT	TACAAGCCTC	AGGCATAGGG	GACGAGACAT	ACGTCCTCAAG	ATCCATCTCT	540
TCATCAGAAA	ACATAACAAC	GATGAAAGAA	GGTCGTGAAG	AAGCCTCTAC	AGTGATCTTT	600
GGAGCACTAG	ACGAACCTTT	CGAGAAGACA	CGTGTAAAAC	CTAAAGACGT	TGGTGTCCCTT	660
GTGGTTAACT	GTAGCATTTT	CAACCCGACA	CCGTCGTTGT	CCGCAATGGT	GATAAACCAT	720
TACAAGATGA	GAGGGAACAT	ACTTAGTTAC	AACCTTGGAG	GGATGGGATG	TTCGGCTGGA	780
ATCATAGCTA	TTGATCTTGC	TCGTGACATG	CTTCAGTCTA	ACCTTAATAG	TTATGCTGTT	840
GTTGTGAGTA	CTGAGATGGT	TGGGTATAAT	TGGTACGTGG	GAAGTGACAA	GTCAATGGTT	900
ATACCTAATT	GTTCCTTTAG	GATGGGTTGT	TCTGCCGTTA	TGCTCTCTAA	CCGTCGTCGT	960
GACTTTGCC	ATGCTAAGTA	CCGTCGAG	CACATTGTC	GAACATCAA	GGCTGCTGAC	1020
GACCGTAGCT	TCAGGAGTGT	GTACCAGGAA	GAAGATGAAC	AAGGATTCAA	GGGGTTGAAG	1080
ATAAGTAGAG	ACTTAATGGA	AGTTGGAGGT	GAAGCTCTCA	AGACAAACAT	CACTACCTTA	1140
GGTCCTCTTG	TCCTACCTT	CTCCGAGCAG	CTTCTCTTCT	TTGCTGCTTT	GGTCGCCGA	1200
ACATTCTCAC	CTGCTGCCAA	AACGTCCACA	ACCACTTC	TCTCTACTTC	CGCCACCGCA	1260
AAAACCAATG	GAATCAAGTC	TTCCCTTCC	GATCTGTC	AGCCATACAT	CCCGGACTAC	1320
AAGCTGCC	TCGAGCATTT	TTGCTTCCAC	CGGGCAAGCA	AAAGTAGTGT	TGAAGAGCTT	1380
CAAAAGAAC	TAGGCTTGAG	TGAAGAGAAAT	ATGGAGGCTT	CTAGGATGAC	ACTTCACAGG	1440
TTTGGAAACA	CTTCTAGCAG	TGGAATCTGG	TATGAGTTGG	CTTACATGGA	GGCCAAGGAA	1500
AGTGTTCGTA	GAGGGATAG	GGTTTGGCAG	ATCGTTTCG	GTTCTGGTTT	TAAGTGTAA	1560
AGTGTGGTGT	GGAAAGGCAAT	GAGGAAGGTG	AAGAAGCCAA	CCAGGAACAA	TCCCTGGGTG	1620
GATTGCATCA	ACCGTTACCC	TGTGCTCTC				1650

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 550 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Gly	Arg	Ser	Asn	Glu	Gln	Asp	Leu	Leu	Ser	Thr	Glu	Ile	Val	Asn
1								10					15		
Arg	Gly	Ile	Glu	Pro	Ser	Gly	Pro	Asn	Ala	Gly	Ser	Pro	Thr	Phe	Ser
								20				25		30	
Val	Arg	Val	Arg	Arg	Arg	Leu	Pro	Asp	Phe	Leu	Gln	Ser	Val	Asn	Leu
								35				40		45	
Lys	Tyr	Val	Lys	Leu	Gly	Tyr	His	Tyr	Leu	Ile	Asn	His	Ala	Val	Tyr
								50				55		60	
Leu	Ala	Thr	Ile	Pro	Val	Leu	Val	Leu	Val	Phe	Ser	Ala	Glu	Val	Gly
								65				70		75	
Ser	Leu	Ser	Arg	Glu	Glu	Ile	Trp	Lys	Lys	Leu	Trp	Asp	Tyr	Asp	Leu
								85				90		95	
Ala	Thr	Val	Ile	Gly	Phe	Phe	Gly	Val	Phe	Val	Leu	Thr	Ala	Cys	Val
								100				105		110	

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Tyr Phe Met Ser Arg Pro Arg Ser Val Tyr Leu Ile Asp Phe Ala Cys
 115 120 125
 Tyr Lys Pro Ser Asp Glu His Lys Val Thr Lys Glu Glu Phe Ile Glu
 130 135 140
 Leu Ala Arg Lys Ser Gly Lys Phe Asp Glu Glu Thr Leu Gly Phe Lys
 145 150 155 160
 Lys Arg Ile Leu Gln Ala Ser Gly Ile Gly Asp Glu Thr Tyr Val Pro
 165 170 175
 Arg Ser Ile Ser Ser Ser Glu Asn Ile Thr Thr Met Lys Glu Gly Arg
 180 185 190
 Glu Glu Ala Ser Thr Val Ile Phe Gly Ala Leu Asp Glu Leu Phe Glu
 195 200 205
 Lys Thr Arg Val Lys Pro Lys Asp Val Gly Val Leu Val Val Asn Cys
 210 215 220
 Ser Ile Phe Asn Pro Thr Pro Ser Leu Ser Ala Met Val Ile Asn His
 225 230 235 240
 Tyr Lys Met Arg Gly Asn Ile Leu Ser Tyr Asn Leu Gly Gly Met Gly
 245 250 255
 Cys Ser Ala Gly Ile Ile Ala Ile Asp Leu Ala Arg Asp Met Leu Gln
 260 265 270
 Ser Asn Pro Asn Ser Tyr Ala Val Val Val Ser Thr Glu Met Val Gly
 275 280 285
 Tyr Asn Trp Tyr Val Gly Ser Asp Lys Ser Met Val Ile Pro Asn Cys
 290 295 300
 Phe Phe Arg Met Gly Cys Ser Ala Val Met Leu Ser Asn Arg Arg Arg
 305 310 315 320
 Asp Phe Arg His Ala Lys Tyr Arg Leu Glu His Ile Val Arg Thr His
 325 330 335
 Lys Ala Ala Asp Asp Arg Ser Phe Arg Ser Val Tyr Gln Glu Glu Asp
 340 345 350
 Glu Gln Gly Phe Lys Gly Leu Lys Ile Ser Arg Asp Leu Met Glu Val
 355 360 365
 Gly Gly Glu Ala Leu Lys Thr Asn Ile Thr Thr Leu Gly Pro Leu Val
 370 375 380
 Leu Pro Phe Ser Glu Gln Leu Leu Phe Phe Ala Ala Leu Val Arg Arg
 385 390 395 400
 Thr Phe Ser Pro Ala Ala Lys Thr Ser Thr Thr Ser Phe Ser Thr
 405 410 415
 Ser Ala Thr Ala Lys Thr Asn Gly Ile Lys Ser Ser Ser Asp Leu
 420 425 430
 Ser Lys Pro Tyr Ile Pro Asp Tyr Lys Leu Ala Phe Glu His Phe Cys
 435 440 445
 Phe His Ala Ala Ser Lys Val Val Leu Glu Glu Leu Gln Lys Asn Leu
 450 455 460
 Gly Leu Ser Glu Glu Asn Met Glu Ala Ser Arg Met Thr Leu His Arg
 465 470 475 480
 Phe Gly Asn Thr Ser Ser Ser Gly Ile Trp Tyr Glu Leu Ala Tyr Met
 485 490 495
 Glu Ala Lys Glu Ser Val Arg Arg Gly Asp Arg Val Trp Gln Ile Ala
 500 505 510
 Phe Gly Ser Gly Phe Lys Cys Asn Ser Val Val Trp Lys Ala Met Arg
 515 520 525
 Lys Val Lys Lys Pro Thr Arg Asn Asn Pro Trp Val Asp Cys Ile Asn
 530 535 540
 Arg Tyr Pro Val Pro Leu
 545 550

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1611 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Genomic DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCGAGCTACG	TCAGGGCTTT	TATATGCACA	AATTCTCATA	AAGTTTCAA	TTTTATTCCA	60
TTTTCTCGG	AAGCCATGGA	AGCTGCTAAT	GAGCCTGTTA	ATGGCGGATC	CGTACAGATC	120
CGAACAGAGA	ACAACGAAAG	ACGAAAGCTT	CCTAACTTCT	TACAAAGCGT	CAACATGAAA	180
TACGTCAAGC	TAGTTATCA	TTACCTCATT	ACTCATCTCT	TCAAGCTCTG	TTGGTTCCA	240
TTAATGGCGG	TTTAGTCAC	AGAGATCTCT	CGATTAACAA	CAGACGATCT	TTACCAAGATT	300
TGGCTTCATC	TCCAAATACAA	TCTCGTTGCT	TTCATCTTTC	TCTCTGCTTT	AGCTATCTT	360
GGCTCCACCG	TTTACATCAT	GAGTCGTCCC	AGATCTGTTT	ATCTCGTTGA	TTACTCTTGT	420
TATCTTCCTC	CGGAGAGTCT	TCAGGTTAAG	TATCAGAAAGT	TTATGGATCA	TTCTAAGTTG	480
ATTGAAGATT	TCAATGAGTC	ATCTTATAGAG	TTTCAGAGGA	AGATCTTGA	ACGTTCTGGT	540
TTAGGAGAAG	AGACTTATCT	CCCTGAAGCT	TTACATTGTA	TCCCTCCGAG	GCCTACGATG	600
ATGGCGGCTC	GTGAGGAATC	TGAGCAGGTA	ATGTTGGTG	CTCTTGATAA	GCTTTTCGAG	660
AATACCAAGA	TAAACCTAG	GGATATTGCT	GTGTTGTTG	TGAATTGTTAG	CTTGTAAAT	720
CCTACACCTT	CGTGTGTCAGC	TATGATTGTT	AACAAAGTATA	AGCTTAGGAG	GAATGTTAAG	780
AGTTTAAACC	TTGGTGGAAAT	GGGGTGTAGT	GCTGGTGTAA	TCTCTATCGA	TTAGCTAA	840
GATATGTTGC	AAGTTCATAG	GAATACTTAT	GCTGTTGTGG	TTAGTACTGA	GAACATTACT	900
CAGAATTGGT	ATTTTGGAA	TAAGAAGGCT	ATGTTGATTG	CGAATTGTTT	GTTTCGTGTT	960
GGTGGTTCGG	CGATTGGTT	GTCGAACAAG	GGGAAAGATC	GTAGACGGTC	TAAGTATAAG	1020
CTTGTTCATA	CCGTTAGGAC	TCATAAAAGGA	GCTGTTGAGA	AGGCTTCAA	CTGTGTTTAC	1080
CAAGACCAAG	ATGATAATGG	GAAGACGGG	GTTGGTGTG	CGAAAGATCT	TATGGCTATA	1140
GCTGGGGAAG	CTCTTAAGGC	GAATATCACT	ACTTTAGGTC	CTTGGTTCT	TCCTATAAGT	1200
GAGCAGATTG	TGTTTTICAT	GACTTTGGTT	ACGAAGAAC	TGTTTAACTC	GAAGCTGAAG	1260
CCGTATATTC	CGGATTCAA	GCTTGCCTT	GATCATTCT	GTATCCATGC	TGGTGGTAGA	1320
GCTGTGATTG	ATGAGCTTGA	GAAGAACTG	CAGCTTCGC	AGACTCATGT	CGAGGCATCC	1380
AGAATGACAC	TGCAACAGATT	TGGAAACACT	TCTTCGAGCT	CGATTTGGTA	TGAACCTGGCT	1440
TACATAGAGG	CTAAAGGTAG	GATGAAGAAA	GGAAACCGGG	TTTGGCAGAT	TGCTTTTGGA	1500
AGTGGGTTA	AGTGTAAACAG	TGCACTTGG	GTGGCTCTAA	ACAATGTCAA	GCCTTCGGTT	1560
AGTAGTCCGT	GGGAACACTG	CATCGACCGA	TATCCGGTTA	AGCTCGACTT	C	1611

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 537 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser	Ser	Tyr	Val	Arg	Ala	Phe	Ile	Cys	Thr	Asn	Ser	His	Lys	Val	Phe
1		5				10						15			
Asn	Phe	Ile	Pro	Phe	Phe	Ser	Glu	Ala	Met	Glu	Ala	Ala	Asn	Glu	Pro
						20			25			30			
Val	Asn	Gly	Gly	Ser	Val	Gln	Ile	Arg	Thr	Glu	Asn	Asn	Glu	Arg	Arg
						35		40			45				
Lys	Leu	Pro	Asn	Phe	Leu	Gln	Ser	Val	Asn	Met	Lys	Tyr	Val	Lys	Leu
						50		55		60					
Gly	Tyr	His	Tyr	Leu	Ile	Thr	His	Leu	Phe	Lys	Leu	Cys	Leu	Val	Pro
65						70			75			80			
Leu	Met	Ala	Val	Leu	Val	Thr	Glu	Ile	Ser	Arg	Leu	Thr	Thr	Asp	Asp
						85		90			95				
Leu	Tyr	Gln	Ile	Trp	Leu	His	Leu	Gln	Tyr	Asn	Leu	Val	Ala	Phe	Ile
						100		105			110				
Phe	Leu	Ser	Ala	Leu	Ala	Ile	Phe	Gly	Ser	Thr	Val	Tyr	Ile	Met	Ser
						115		120			125				
Arg	Pro	Arg	Ser	Val	Tyr	Leu	Val	Asp	Tyr	Ser	Cys	Tyr	Leu	Pro	Pro
						130		135			140				
Glu	Ser	Leu	Gln	Val	Lys	Tyr	Gln	Lys	Phe	Met	Asp	His	Ser	Lys	Leu
145						150			155			160			
Ile	Glu	Asp	Phe	Asn	Glu	Ser	Ser	Leu	Glu	Phe	Gln	Arg	Lys	Ile	Leu
						165			170			175			
Glu	Arg	Ser	Gly	Leu	Gly	Glu	Glu	Thr	Tyr	Leu	Pro	Glu	Ala	Leu	His
						180			185			190			

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Cys Ile Pro Pro Arg Pro Thr Met Met Ala Ala Arg Glu Glu Ser Glu
 195 200 205
 Gln Val Met Phe Gly Ala Leu Asp Lys Leu Phe Glu Asn Thr Lys Ile
 210 215 220
 Asn Pro Arg Asp Ile Gly Val Leu Val Val Asn Cys Ser Leu Phe Asn
 225 230 235 240
 Pro Thr Pro Ser Leu Ser Ala Met Ile Val Asn Lys Tyr Lys Leu Arg
 245 250 255
 Gly Asn Val Lys Ser Phe Asn Leu Gly Gly Met Gly Cys Ser Ala Gly
 260 265 270
 Val Ile Ser Ile Asp Leu Ala Lys Asp Met Leu Gln Val His Arg Asn
 275 280 285
 Thr Tyr Ala Val Val Ser Thr Glu Asn Ile Thr Gln Asn Trp Tyr
 290 295 300
 Phe Gly Asn Lys Ala Met Leu Ile Pro Asn Cys Leu Phe Arg Val
 305 310 315 320
 Gly Gly Ser Ala Ile Leu Leu Ser Asn Lys Gly Lys Asp Arg Arg Arg
 325 330 335
 Ser Lys Tyr Lys Leu Val His Thr Val Arg Thr His Lys Gly Ala Val
 340 345 350
 Glu Lys Ala Phe Asn Cys Val Tyr Gln Glu Gln Asp Asp Asn Gly Lys
 355 360 365
 Thr Gly Val Ser Leu Ser Lys Asp Leu Met Ala Ile Ala Gly Glu Ala
 370 375 380
 Leu Lys Ala Asn Ile Thr Thr Leu Gly Pro Leu Val Leu Pro Ile Ser
 385 390 395 400
 Glu Gln Ile Leu Phe Phe Met Thr Leu Val Thr Lys Lys Leu Phe Asn
 405 410 415
 Ser Lys Leu Lys Pro Tyr Ile Pro Asp Phe Lys Leu Ala Phe Asp His
 420 425 430
 Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Glu Leu Glu Lys
 435 440 445
 Asn Leu Gln Leu Ser Gln Thr His Val Glu Ala Ser Arg Met Thr Leu
 450 455 460
 His Arg Phe Gly Asn Thr Ser Ser Ser Ile Trp Tyr Glu Leu Ala
 465 470 475 480
 Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Arg Val Trp Gln
 485 490 495
 Ile Ala Phe Gly Ser Gly Phe Lys Cys Asn Ser Ala Val Trp Val Ala
 500 505 510
 Leu Asn Asn Val Lys Pro Ser Val Ser Ser Pro Trp Glu His Cys Ile
 515 520 525
 Asp Arg Tyr Pro Val Lys Leu Asp Phe
 530 535

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1502 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCTCCGACGA	TGCCTCAGGC	ACCGATGCCA	GAGTTCTCTA	GCTCGGTGAA	GCTCAAGTAC	60
GTGAAACTTG	GTTACCAATA	TTTGGTIAAC	CATTTCCTGA	GTTTCTTTT	GATCCCGATC	120
ATGGCTATTG	TCGCCGTTGA	GCTTCTTCGG	ATGGGTCTCG	AAGAGATCCT	TAATGTTTGG	180
AATTCACTCC	AGTTTGACCT	AGTTCAAGTT	CTATGTTCTT	CCTTCTTTGT	CATCTTCATC	240
TCCACTGTTT	ACTTCATGTC	CAAGCCACGC	ACCATCTACC	TCGTTGACTA	TTCTTGTGAC	300
AAGCCACCTG	TCACGTGTCG	TGTCCCCCTTC	GCAACTTCA	TGGAACACTC	TCGTTTGATC	360
CTCAAGGACA	ACGCCAACGAG	CGTCGAGTT	CAAATGAGAA	TCCTTGAACG	TTCTGGCCTC	420
GGTGAGGAGA	CTTGCTCTCCC	TCCGGCTATT	CATTATATT	CTCCACACC	AACCATGGAC	480
GCGGCTAGAA	GCGAGGCTCA	GATGGTTATC	TTCGAGGCCA	TGGACGATCT	TTTCAAGAAA	540
ACCGGTCTTA	AACCTAAAGA	CGTCGACATC	CTTATCGTCA	ACTGCTCTCT	TTTCTCTCCC	600

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ACACCATCGC	TCTCAGCTAT	GGTCATCAAC	AAATATAAGC	TTAGGAGTAA	TATCAAGAGC	660
TTCAATCTT	CGGGGATGGG	CTGCAGCGCG	GGCCTGATCT	CAGTTGATCT	AGCCCGCGAC	720
TTGCTCCAAG	TTCATCCAA	TTCAAATGCA	ATCATCGTC	GCACGGAGAT	CATAACGCT	780
AATTACTATC	AAGGCAACGA	GAGAGCCATG	TTGTTACCCA	ATTGTCTCTT	CCGCATGGGT	840
GCGGCAGCCA	TACACATGTC	AAACCGCCGG	TCTGACCGGT	GGCGAGCCAA	ATACAAGCTT	900
TCCCACCTCG	TCCGGACACA	CCGTGGCGCT	GACGACAAGT	CTTCTACTG	TGTCTACGAA	960
CAGGAAGACA	AAGAAGGACA	CGITGGCATC	AACTTGTCCA	AAGATCTCAT	GGCCATCGCC	1020
GGTGAAGCCC	TCAAGGCAA	CATCACCACA	ATAGGTCTT	TGGTCCTTAC	GGCGTCAGAA	1080
CAACTTCTCT	TCCTCACGTC	CCTAATCGGA	CGTAAAATCT	TCAACCCGAA	ATGGAAACCA	1140
TACATACCGG	ATTCTCAAGCT	GGCCTTCGAA	CACTTTGCA	TTCACGCAGG	AGGCAGAGCG	1200
GTGATCGACG	AGCTCCAAAA	GAATCTACAA	CTATCAGGAG	AACACGTTGA	GGCCTCAAGA	1260
ATGACACTAC	ATCGTTTTGG	TAACACGTC	TCTTCATCGT	TATGGTACGA	GCTTAGCTAC	1320
ATCGAGTCTA	AAGGGAGAAAT	GAGGAGAGGC	GATCGCCTTT	GGCAAATCGC	GTTTGGGAGT	1380
GGTTTCAAGT	GTAACTCTGC	CGTGTGGAAG	TGTAACCGTA	CGATTAAGAC	ACCTAAGGAC	1440
GGACCATGGT	CCGATTGTAT	CGACCGTTAC	CCTGTCTTA	TTCCCGAAGT	TGTCAAACTC	1500
TA						1502

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser	Pro	Thr	Met	Pro	Gln	Ala	Pro	Met	Pro	Glu	Phe	Ser	Ser	Ser	Val
1			5					10				15			
Lys	Leu	Lys	Tyr	Val	Lys	Leu	Gly	Tyr	Gln	Tyr	Leu	Val	Asn	His	Phe
								20			25		30		
Leu	Ser	Phe	Leu	Leu	Ile	Pro	Ile	Met	Ala	Ile	Val	Ala	Val	Glu	Leu
								35			40		45		
Leu	Arg	Met	Gly	Pro	Glu	Glu	Ile	Leu	Asn	Val	Trp	Asn	Ser	Leu	Gln
								50			55		60		
Phe	Asp	Leu	Val	Gln	Val	Leu	Cys	Ser	Ser	Phe	Phe	Val	Ile	Phe	Ile
								65			70		75		80
Ser	Thr	Val	Tyr	Phe	Met	Ser	Lys	Pro	Arg	Thr	Ile	Tyr	Leu	Val	Asp
								85			90		95		
Tyr	Ser	Cys	Tyr	Lys	Pro	Pro	Val	Thr	Cys	Arg	Val	Pro	Phe	Ala	Thr
								100			105		110		
Phe	Met	Glu	His	Ser	Arg	Leu	Ile	Leu	Lys	Asp	Lys	Pro	Lys	Ser	Val
								115			120		125		
Glu	Phe	Gln	Met	Arg	Ile	Leu	Glu	Arg	Ser	Gly	Leu	Gly	Glu	Glu	Thr
								130			135		140		
Cys	Leu	Pro	Pro	Ala	Ile	His	Tyr	Ile	Pro	Pro	Thr	Pro	Thr	Met	Asp
								145			150		155		160
Ala	Ala	Arg	Ser	Glu	Ala	Gln	Met	Val	Ile	Phe	Glu	Ala	Met	Asp	Asp
								165			170		175		
Leu	Phe	Lys	Lys	Thr	Gly	Leu	Lys	Pro	Lys	Asp	Val	Ile	Leu	Ile	
								180			185		190		
Val	Asn	Cys	Ser	Leu	Phe	Ser	Pro	Thr	Pro	Ser	Leu	Ser	Ala	Met	Val
								195			200		205		
Ile	Asn	Lys	Tyr	Lys	Leu	Arg	Ser	Asn	Ile	Lys	Ser	Phe	Asn	Leu	Ser
								210			215		220		
Gly	Met	Gly	Cys	Ser	Ala	Gly	Leu	Ile	Ser	Val	Asp	Leu	Ala	Arg	Asp
								225			230		235		240
Leu	Leu	Gln	Val	His	Pro	Asn	Ser	Asn	Ala	Ile	Ile	Val	Ser	Thr	Glu
								245			250		255		
Ile	Ile	Thr	Pro	Asn	Tyr	Tyr	Gln	Gly	Asn	Glu	Arg	Ala	Met	Leu	Leu
								260			265		270		
Pro	Asn	Cys	Leu	Phe	Arg	Met	Gly	Ala	Ala	Ala	Ile	His	Met	Ser	Asn
								275			280		285		
Arg	Arg	Ser	Asp	Arg	Trp	Arg	Ala	Lys	Tyr	Lys	Leu	Ser	His	Leu	Val
								290			295		300		

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Arg Thr His Arg Gly Ala Asp Asp Lys Ser Phe Tyr Cys Val Tyr Glu
 305 310 315 320
 Gln Glu Asp Lys Glu Gly His Val Gly Ile Asn Leu Ser Lys Asp Leu
 325 330 335
 Met Ala Ile Ala Gly Glu Ala Leu Lys Ala Asn Ile Thr Thr Ile Gly
 340 345 350
 Pro Leu Val Leu Pro Ala Ser Glu Gln Leu Leu Phe Leu Thr Ser Leu
 355 360 365
 Ile Gly Arg Lys Ile Phe Asn Pro Lys Trp Lys Pro Tyr Ile Pro Asp
 370 375 380
 Phe Lys Leu Ala Phe Glu His Phe Cys Ile His Ala Gly Gly Arg Ala
 385 390 395 400
 Val Ile Asp Glu Leu Gln Lys Asn Leu Gln Leu Ser Gly Glu His Val
 405 410 415
 Glu Ala Ser Arg Met Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser
 420 425 430
 Ser Leu Trp Tyr Glu Leu Ser Tyr Ile Glu Ser Lys Gly Arg Met Arg
 435 440 445
 Arg Gly Asp Arg Val Trp Gln Ile Ala Phe Gly Ser Gly Phe Lys Cys
 450 455 460
 Asn Ser Ala Val Trp Lys Cys Asn Arg Thr Ile Lys Thr Pro Lys Asp
 465 470 475 480
 Gly Pro Trp Ser Asp Cys Ile Asp Arg Tyr Pro Val Phe Ile Pro Glu
 485 490 495
 Val Val Lys Leu
 500

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1548 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGGACGGTG	CCGGAGAACTC	ACGACTCGGT	GGTGATGGTG	GTGGTGTGATGG	TTCTGTTGGA	60
GTTCAGATCC	GACAAACACG	GATGCTACCG	GATTTCTCC	AGAGCGTGA	TCTCAAGTAT	120
GTGAAATTAG	GTTACCATTA	CTTAATCTCA	AATCTCTGA	CTCTCTGTTT	ATTCCTCTC	180
GCCGTTGITA	TCTCCGTCGA	AGCCTCTCA	ATGAACCCAG	ATGATCTCAA	ACAGCTCTGG	240
ATCCATCTAC	AATAACAATCT	GGTTAGTATC	ATCATCTGTT	CAGCGATTCT	AGTCITTCGGG	300
TTAACGGTTT	ATGTTATGAC	CCGACCTAGA	CCCGTTACT	TGGTTGATTT	CTCTTGTAT	360
CTCCCCACCTG	ATCATCTCAA	AGCTCCTTAC	GCTCGGGTCA	TGGAACATT	TAGACTCACC	420
GGAGATTTCG	ATGACTTGC	TCTCGAGTTC	CAACCGAAGA	TCCTTGAGCG	TTCTGGTTTA	480
GGGGAAGACA	CTTATGTCCC	TGAAGCTATG	CATTATGTT	CACCGAGAAT	TTCAATGGCT	540
GCTGCTAGAG	AAGAAGCTGA	ACAAGCTATG	TTTGGTGTCT	TAGATAACCT	TTTCGCTAAC	600
ACTAATGTGA	AACCAAAGGA	TATTGGAATC	CTTGGTTGTGA	ATTGTTAGTCT	CTTTAATCCA	660
ACTCCCTTCGT	TATCTGCAAT	GATTGTGAAC	AAGTATAAGC	TTAGAGGTAA	CATTAGAAC	720
TACAATCTAG	GCGGTATGGG	TTGCAGCGCG	GGAGTTATCG	CTGTTGATCT	TGCTAAAGAC	780
ATGTTGTTGG	TACATAGGAA	CACTTATGCG	GTTGTTGTTT	CTACTGAGAA	CATTACTCAG	840
AATTGGTATT	TTGGTACCAA	GAAATCGATG	TTGATACCGA	ACTGCTTGT	TCGAGTTGGT	900
GGCTCTCGGG	TTTGTCTATC	GAACAAAGTCG	AGGGACAAAGA	GACGGTCTAA	GTACAGGCTT	960
GTACATGTAG	TCAGGACTCA	CCGTGGAGCA	GATGATAAAG	CTTTCGTTG	TGTTTATCAA	1020
GAGCAGGATG	ATACAGGGAG	AACCGGGGTT	TCGTTGTCGA	AAGATCTAAT	GGCGATTGCA	1080
GGGGAAACTC	TCAAAACCAA	TATCACTACA	TTGGGTCTC	TTGTTCTACC	GATAAGTGAG	1140
CAGATTCTCT	TCTTATGAC	TCTAGTTGTC	AAGAAGCTCT	TTAACGGTAA	AGTGAAACCG	1200
TATATCCCGG	ATTTCAAACT	TGCTTTGAG	CATTTCGTA	TCCATGCTGG	TGGAAGAGCT	1260
GTGATCGATG	AGTTAGAGAA	GAATCTGCA	CTTTCACCA	TTCATGTCGA	GGCTTCGAGG	1320
ATGACTCTTC	ATCGATTGG	TAACACATCT	TCGAGCTCCA	TTTGGTATGA	ATTGGCTTAC	1380
ATTGAACCGA	AGGGAAGGGAT	GCGAAGAGGT	AATCGTCTT	GGCAAATCGC	GTTCGGAAGT	1440
GGATTTAAAT	GTAATAGCGC	GATTTGGGAA	GCATTAAGGC	ATGTGAAACC	TCGAACAAC	1500
AGTCCTGGG	AAGATTGTAT	TGACAAGTAT	CCGGTAACCT	TAAGTTAT		1548

(2) INFORMATION FOR SEQ ID NO:14:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 516 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Asp Gly Ala Gly Glu Ser Arg Leu Gly Gly Asp Gly Gly Asp
 1 5 10 15
 Gly Ser Val Gly Val Gln Ile Arg Gln Thr Arg Met Leu Pro Asp Phe
 20 25 30
 Leu Gln Ser Val Asn Leu Lys Tyr Val Lys Leu Gly Tyr His Tyr Leu
 35 40 45
 Ile Ser Asn Leu Leu Thr Leu Cys Leu Phe Pro Leu Ala Val Val Ile
 50 55 60
 Ser Val Glu Ala Ser Gln Met Asn Pro Asp Asp Leu Lys Gln Leu Trp
 65 70 75 80
 Ile His Leu Gln Tyr Asn Leu Val Ser Ile Ile Ile Cys Ser Ala Ile
 85 90 95
 Leu Val Phe Gly Leu Thr Val Tyr Val Met Thr Arg Pro Arg Pro Val
 100 105 110
 Tyr Leu Val Asp Phe Ser Cys Tyr Leu Pro Pro Asp His Leu Lys Ala
 115 120 125
 Pro Tyr Ala Arg Phe Met Glu His Ser Arg Leu Thr Gly Asp Phe Asp
 130 135 140
 Asp Ser Ala Leu Glu Phe Gln Arg Lys Ile Leu Glu Arg Ser Gly Leu
 145 150 155 160
 Gly Glu Asp Thr Tyr Val Pro Glu Ala Met His Tyr Val Pro Pro Arg
 165 170 175
 Ile Ser Met Ala Ala Ala Arg Glu Glu Ala Glu Gln Val Met Phe Gly
 180 185 190
 Ala Leu Asp Asn Leu Phe Ala Asn Thr Asn Val Lys Pro Lys Asp Ile
 195 200 205
 Gly Ile Leu Val Val Asn Cys Ser Leu Phe Asn Pro Thr Pro Ser Leu
 210 215 220
 Ser Ala Met Ile Val Asn Lys Tyr Lys Leu Arg Gly Asn Ile Arg Ser
 225 230 235 240
 Tyr Asn Leu Gly Gly Met Gly Cys Ser Ala Gly Val Ile Ala Val Asp
 245 250 255
 Leu Ala Lys Asp Met Leu Leu Val His Arg Asn Thr Tyr Ala Val Val
 260 265 270
 Val Ser Thr Glu Asn Ile Thr Gln Asn Trp Tyr Phe Gly Asn Lys Lys
 275 280 285
 Ser Met Leu Ile Pro Asn Cys Leu Phe Arg Val Gly Gly Ser Ala Val
 290 295 300
 Leu Leu Ser Asn Lys Ser Arg Asp Lys Arg Arg Ser Lys Tyr Arg Leu
 305 310 315 320
 Val His Val Val Arg Thr His Arg Gly Ala Asp Asp Lys Ala Phe Arg
 325 330 335
 Cys Val Tyr Gln Glu Gln Asp Asp Thr Gly Arg Thr Gly Val Ser Leu
 340 345 350
 Ser Lys Asp Leu Met Ala Ile Ala Gly Glu Thr Leu Lys Thr Asn Ile
 355 360 365
 Thr Thr Leu Gly Pro Leu Val Leu Pro Ile Ser Glu Gln Ile Leu Phe
 370 375 380
 Phe Met Thr Leu Val Val Lys Lys Leu Phe Asn Gly Lys Val Lys Pro
 385 390 395 400
 Tyr Ile Pro Asp Phe Lys Leu Ala Phe Glu His Phe Cys Ile His Ala
 405 410 415
 Gly Gly Arg Ala Val Ile Asp Glu Leu Glu Lys Asn Leu Gln Leu Ser
 420 425 430
 Pro Val His Val Glu Ala Ser Arg Met Thr Leu His Arg Phe Gly Asn
 435 440 445

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Thr Ser Ser Ser Ile Trp Tyr Glu Leu Ala Tyr Ile Glu Ala Lys
450 455 460
Gly Arg Met Arg Arg Gly Asn Arg Val Trp Gln Ile Ala Phe Gly Ser
465 470 475 480
Gly Phe Lys Cys Asn Ser Ala Ile Trp Glu Ala Leu Arg His Val Lys
485 490 495
Pro Ser Asn Asn Ser Pro Trp Glu Asp Cys Ile Asp Lys Tyr Pro Val
500 505 510
Thr Leu Ser Tyr
515

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WHAT IS CLAIMED IS:

1. An isolated polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an amino acid sequence substantially identical to SEQ ID NO:14.
2. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:2.
3. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:4.
4. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:6.
5. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:8.
6. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:10.
7. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:12.
8. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:14.

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9. An isolated polynucleotide, wherein said polynucleotide is selected from the group consisting of:

- a) SEQ ID NO:1;
- b) SEQ ID NO:3;
- c) SEQ ID NO:5;
- d) SEQ ID NO:7;
- e) SEQ ID NO:9;
- f) SEQ ID NO:11;
- g) SEQ ID NO:13;
- h) an RNA analog of SEQ ID NO:1;
- i) an RNA analog of SEQ ID NO:3;
- j) an RNA analog of SEQ ID NO:5;
- k) an RNA analog of SEQ ID NO:7;
- l) an RNA analog of SEQ ID NO:9;
- m) an RNA analog of SEQ ID NO:11;
- n) an RNA analog of SEQ ID NO:13;
- o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k), l), m), or n); and
- p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

10. An isolated polypeptide having an amino acid sequence selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an

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amino acid sequence substantially identical to SEQ ID NO:14.

11. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:2.

12. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:4.

13. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:6.

14. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:8.

15. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:10.

16. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:12.

17. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:14.

18. A transgenic plant containing a nucleic acid construct comprising a polynucleotide selected from the group consisting of:

- a) SEQ ID NO:1;
- b) SEQ ID NO:3;
- c) SEQ ID NO:5;
- d) SEQ ID NO:7;
- e) SEQ ID NO:9;
- f) SEQ ID NO:11;
- g) SEQ ID NO:13;
- h) an RNA analog of SEQ ID NO:1;

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- i) an RNA analog of SEQ ID NO:3;
- j) an RNA analog of SEQ ID NO:5;
- k) an RNA analog of SEQ ID NO:7;
- l) an RNA analog of SEQ ID NO:9;
- m) an RNA analog of SEQ ID NO:11;
- n) an RNA analog of SEQ ID NO:13;
- o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k), l), m), or n); and
- p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

19. The plant of claim 18, wherein said construct further comprises a regulatory element operably linked to said polynucleotide.

20. The plant of claim 19, wherein said regulatory element is a tissue-specific promoter.

21. The plant of claim 20, wherein said regulatory element is an epidermal cell-specific promoter.

22. The plant of claim 20, wherein said regulatory element is a seed-specific promoter that is operably linked in sense orientation to said polynucleotide.

23. The plant of claim 22, wherein said plant has altered levels of very long chain fatty acids in seeds compared to the levels in a plant lacking said nucleic acid construct.

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24. A transgenic plant containing a nucleic acid construct comprising a polynucleotide encoding a polypeptide selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an amino acid sequence substantially identical to SEQ ID NO:14.

25. The plant of claim 24, wherein said construct further comprises a regulatory element operably linked to said polynucleotide.

26. The plant of claim 25, wherein said regulatory element is a tissue-specific promoter.

27. The plant of claim 26, wherein said regulatory element is an epidermal cell-specific promoter.

28. The plant of claim 26, wherein said regulatory element is a seed-specific promoter that is operably linked in sense orientation to said polynucleotide.

29. The plant of claim 28, wherein said plant has altered levels of very long chain fatty acids in seeds compared to the levels in a plant lacking said nucleic acid construct.

30. A method of altering the levels of very long chain fatty acids in a plant, comprising the steps of:

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A) creating a nucleic acid construct, said construct comprising a polynucleotide selected from the group consisting of:

- a) SEQ ID NO:1;
 - b) SEQ ID NO:3;
 - c) SEQ ID NO:5;
 - d) SEQ ID NO:7;
 - e) SEQ ID NO:9;
 - f) SEQ ID NO:11;
 - g) SEQ ID NO:13;
 - h) an RNA analog of SEQ ID NO:1;
 - i) an RNA analog of SEQ ID NO:3;
 - j) an RNA analog of SEQ ID NO:5;
 - k) an RNA analog of SEQ ID NO:7;
 - l) an RNA analog of SEQ ID NO:9;
 - m) an RNA analog of SEQ ID NO:11;
 - n) an RNA analog of SEQ ID NO:13;
 - o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k), l), m), or n); and
 - p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14; and
- B) introducing said construct into said plant, wherein said polynucleotide is effective for altering the levels of very long chain fatty acids in said plant.

Figure 1

FAE1 w/ respect to time

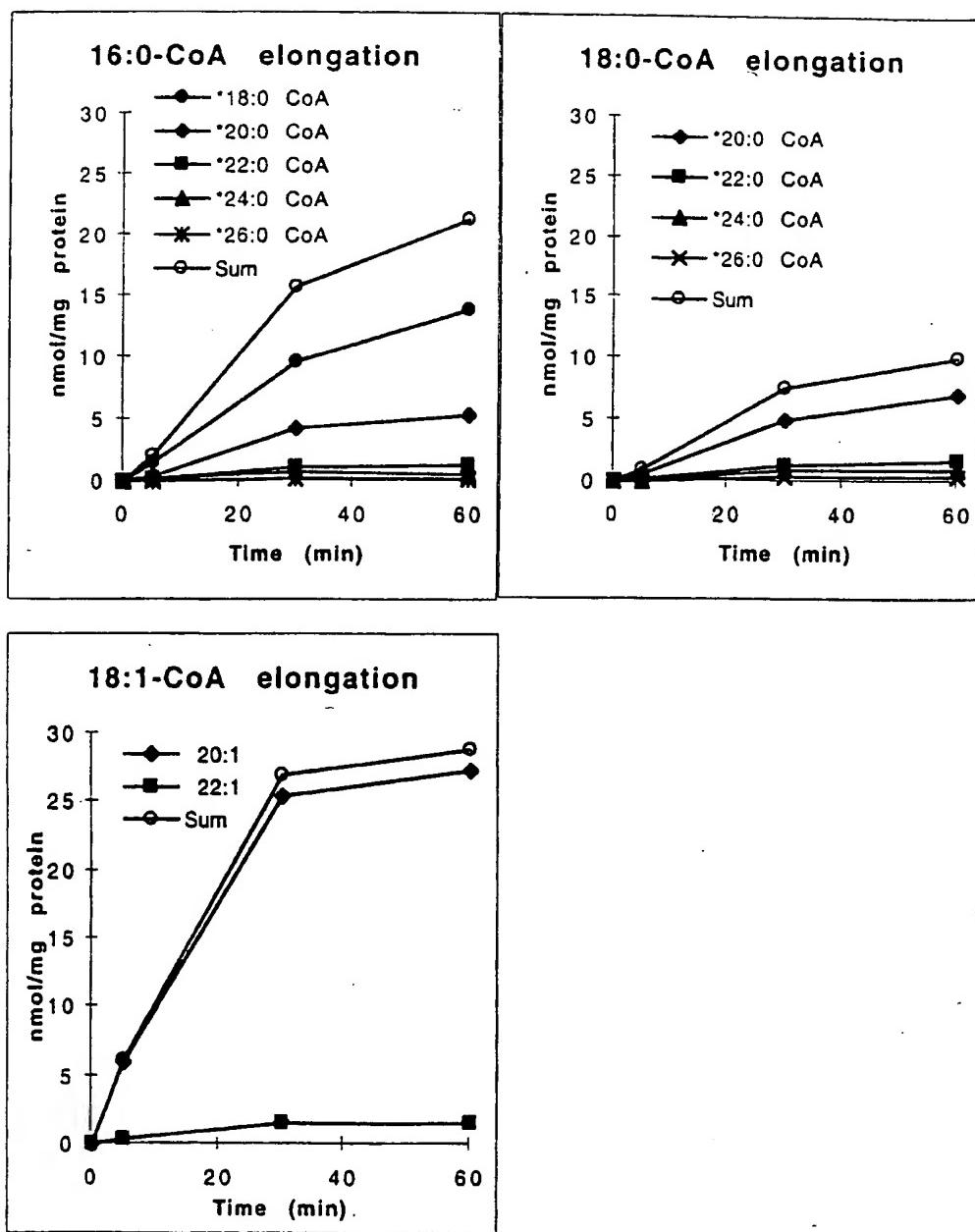
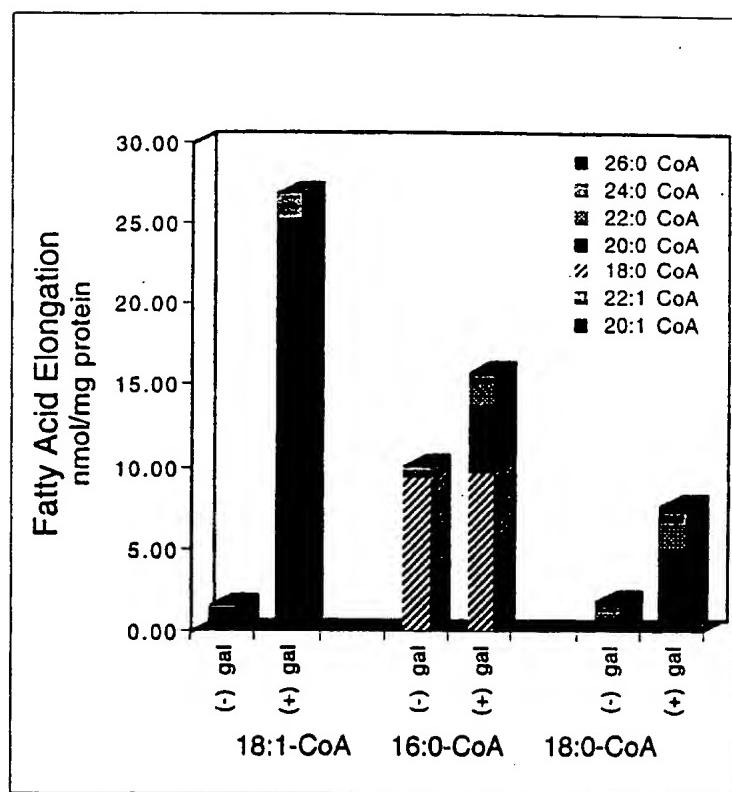


Figure 2



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EL1 1560 bases

ATGGATCGAG AGAGATTAAC GGCGGAGATG GCGTTTCGAG ATTCAATCATC GGCCGTTATA
AGAATTGAA GACGTTGCC GGATTATTAA ACGTCCGTTA AGCTCAAATA CGTGAAGCTT
GGACTTCACA ACTCTTGCAA CGTGACCACC ATTCTCTTCT TCTTAATTAT TCTTCCTTTA
ACCGGAACCG TGCTGGTTCA GCTAACCGGT CTAACGTTCG ATACGTTCTC TGAGCTTGG
TCTAACCAAGG CGGTTCAACT CGACACGGCG ACGAGACTTA CCTGCTTGGT TTTCCCTCTCC
TTCGTTTGAG CCCTCTACGT GGCTAACCGG TCTAAACCGG TTTACCTAGT GGATTTCTCC
TGCTACAAAC CGGAAGACGA GCGTAAAATA TCAGTAGATT CGTTCTTGAC GATGACTGAG
GAAAATGGAT CATTCAACCGA TGACACGGTT CAGTTCCAGC AAAGAATCTC GAACCGGGCC
GGTTTGGGAG ACGAGACGTA TCTGCCACGT GGCTAACTT CAACGGCCCC GAAGCTAAAT
ATGTCAGAGG CACGTGCCGA AGCTGAAGCC GTTATGTTTG GAGCCTTAGA TTCCCTCTTC
GAGAAAACCG GAATTAAACC GGCGAAGTC GGAATCTTGA TAGTAAACTG CAGCTTATTC
AATCCGACGC CGTCTCTATC AGCGATGATC GTGAACCATT ACAAGATGAG AGAAGACATC
AAAAGTTACA ACCTCGGAGG AATGGGTTGC TCCGCCGGAT TAATCTCAAT CGATCTCGCT
ACAATCTCC TCAAAGCAAA CCCTAATTCT TACGCTGTGC TGGTAAGCAC GGAAAACATA
ACCCTAAACT GGTACTTCGG AAATGACCAGG TCAATGCTCC TCTGCAACTG CATCTCCGA
ATGGGCGGAG CTGCGATTCT CCTCTCTAAC CGCGTCAAG ACCGGAAGAA GTCAAAGTAC
TCGCTGGTCA ACGTCGTTCG AACACATAAA GGATCAGACG ACAAGAACTA CAATTGCGTG
TACCAGAAGG AAGACGAGAG AGGAACAATC GGTGTCTCTT TAGCTAGAGA GCTCATGTCT
GTCGCCGGAG ACGCTCTGAA AACAAACATC ACCGACTTTAG GACCGATGGT TCTTCCATTG
TCAGAGCAGT TGATGTTCTT GATTTCTTG GTCAAAAGGA AGATGTTCAA GTTAAAAGTT
AAACCGTATA TTCCGGATT CAAGCTAGCT TTCGAGCATT TCTGTATTCA CGCAGGAGGT
AGAGCGGTTAGACCGAAGT GCAGAAGAAT CTTGATCTCA AAGATTGGCA CATGGAACCT
TCTAGAATGA CTTTGCACAG ATTTGGTAAC ACTTCGAGTA GCTCGCTTTG GTATGAGATG
GCTTATACCG AAGCTAAGGG TCGGGTTAAA GCTGGTGACC GACTTGGCA GATTGCGTTT
GGATCGGGTT TCAAGTGTAA TAGTGCCTT TGAAAGCGT TACGACCGGT TTGACCGGAG
GAGATGACCG GTAATGCTTG GGCTGGTTCA ATTGATCAAT ATCCGGTTAA AGTTGTGCAA

EL1
FIGURE 3

EL1 sequence

Molecular Weight 58379.00 Daltons

520 Amino Acids

62 Strongly Basic(+) Amino Acids (K,R)

52 Strongly Acidic(-) Amino Acids (D,E)

187 Hydrophobic Amino Acids (A,I,L,F,W,V)

144 Polar Amino Acids (N,C,Q,S,T,Y)

8.784 Isoelectric Point

10.804 Charge at PH 7.0

MDRERLTAEM AFRDSSSAVI RIRRLPDLL TSVKLKYVKL GLHNSCNVTT ILFFLIILPL
TGTVLVQLTG LTFTDFSELW SNQAVQLDTA TRLTCLVFLS FVLTLYVANR SKPVYLVDFS
CYKPEDERKI SVDSFLTMTE ENGSFTDDTV QQQQRISNRA GLGDETYLPR GITSTPPKLN
MSEARAEAEA VMFGALDSL EKTGIKPAEV GILIVNCRLF NPTPSLSAMI VNHYKMREDI
KSYNLGGMGC SAGLISIDL NNLLKANPNS YAVVVSTENI TLNWYFGNDR SMLLCNCIFR
MGGAAILLSN RRQDRKKSKY SLVNVRTHK GSDDKNYNVC YQKEDERGTI GVSLARELMS
VAGDALKTNI TTLGPMVLPL SEQLMFLISL VKRKMFKLK KV KPYIPDFKLA FEHFCIHAGG
RAVLDEVQKN LDLKDWHMEP SRMTLHRFGN TSSSSLWYEM AYTEAKGRVK AGDRLWQIAF
GSGFKCNSAV WKALRPVSTE EMTGNAWAGS IDQYPVVKVVQ

FIGURE 4

EL2 1479 bases

ATGGGATTACC	CCATGAAGAA	GGTAAAAATC	TTTTCAACT	ACCTCATGGC	GCATCGCTTC	
AAGCTCTGCT	TCTTACCAATT	AATGGTTGCT	ATAGCGTGG	AGGCGTCTCG	TCTTTCCACA	120
CAAGATCTCC	AAAACTTTA	CCTCTACTTA	CAAACAAACC	ACACATCTCT	AACCATGTTTC	
TCCTTTACC	TCGCTCTCGG	GTCGACTCTT	TACCTCATGA	CCCGGCCCAA	ACCCGTTTAT	240
CTCGTTGACT	TTAGCTGCTA	CCTCCCACCG	TCGCATCTCA	AAGCCAGCAC	CCAGAGGATC	
ATGCAACACG	TAAGGCTTGT	ACGAGAACGCA	GGCGCGTGG	AGCAAGAGTC	CGATTACTTG	360
ATGGACTTCT	GCGAGAACAT	TCTAGAACGT	TCCGGTCTAG	GCCAAGAGAC	GTACGTACCC	
GAAGGTCTTC	AAACTTGCC	ACTAACACAG	AATTGGCTG	TATCACGTAT	AGAGACGGAG	480
GAAGTTATTAA	TTGGTGGCGGT	CGATAATCTG	TTTCGCAACA	CGGGATAAAG	CCCTAGTGAT	
ATAGGTATAT	TGGTGGTGAA	TTCAAGCACT	TTTAATCCAA	CACCTTCGCT	ATCAAGTATC	600
TTAGTGATAA	AGTTTAAACT	TAGGGATAAT	ATAAGAGCT	TGAATCTTGG	TGGGATGGGG	
TGTAGCGCTG	GAGTCATCGC	TATCGATGCG	GCTAAGAGCT	TGTTACAAGT	TCATAGAAC	720
ACTTATGCTC	TTGTGGTGAG	CACGGAGAAC	ATCACTCAA	ACTTGTACAT	GGGTAACAAAC	
AAATCAATGT	TGGTTACAAA	CTGTTTGTTC	CGTATAGGTG	GGGCCGCGAT	TTTGTCTTCT	840
AACCGGTCTA	TAGATCGTAA	ACGCCAAGAA	TACCGAGCTT	TTCACACCGT	GGGGTCCAT	
ACCGGAGCAG	ATGACCGATC	CTATGAATGT	GCAACTCAAG	AAGAGGATGA	AGATGGCATA	960
GTGGGGTTT	CCTTGTCAAA	GAATCTACCA	ATGGTAGCTG	CAAGAACCT	AAAGATCAAT	
ATCGCAACTT	TGGGTCGGCT	TGTTCTTCCC	ATAAGCGAGA	AGTTTCACTT	CTTTGTGAGG	1080
TTCGTTAAAA	AGAAGTTCT	CAACCCCAAG	CTAAAGCATT	ACATTCCGGA	TTTCAAGCTC	
GCATTCGAGC	ATTTCTGTAT	CCATGCGGGT	GTTAGAGCGC	TAATTGATGA	GATGGAGAAG	1200
AATCTTCATC	TAACTCCACT	AGACGTTGAG	GCTTCAAGAA	TGACATTACA	CAGGTTTGGT	
AATACCTCTT	CGAGCTCCAT	TTGGTACGAG	TTGGCTTACA	CAGAACCAA	AGGAAGGATG	1320
ACGAAAGGAG	ATAGGATTG	GCAGATTGCG	TTGGGGTCAG	GTTTTAAGTG	TAATAGTTCA	
GTGGGGTGG	CTCTTCGTA	CGTCAAGCCT	TCTACTAATA	ATCCTGGGA	ACAGTGTCTA	1440
CACAAATATC	CAGTTGAGAT	CGATATAGAT	TTAAAAGAG			

EL2
FIGURE 5

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EL2 protein sequence
Molecular Weight 55799.30 Daltons
493 Amino Acids
55 Strongly Basic(+) Amino Acids (K,R)
46 Strongly Acidic(-) Amino Acids (D,E)
181 Hydrophobic Amino Acids (A,I,L,F,W,V)
134 Polar Amino Acids (N,C,Q,S,T,Y)
8.756 Isoelectric Point
10.995 Charge at PH 7.0

MDYPMKKVKI FFNYLMAHRF KLCFLPLMVA IAVEASRLST QDLQNFYLYL QNNHTSLTMF FLYLALGSTL
YLMTRPKPVY LVDFSCYLPP SHLKASTQRI MQHVRLVREA GAWKQESDYL MDFCEKILER SGLGQETYVP
EGLQTLPLQQ NLAVSRIETE EVIIGAVDNL FRNTGISPSD IGILVVNSST FNPTPSLSSI LVNKFKLRDN
IKSLNLGGMG CSAGVIAIDA AKSLLQVHRN TYALVVSTEM ITQONLYMGNN KSMLVTNCLF RIGGAAILLS
NRSIDRKRAK YELVHTVRVH TGADDRSYEC ATQEEDDEDGI VGVSLSKNLP MVAARTLKIN IATLGPLVLP
ISEKFHFFVR FVKKKFLNPK LKHYPDFKL AFEHFCIHAG GRALIDEMEK NLHLTPLDVE ASRMTLHRFG
NTSSSSIWYE LAYTEAKGRM TKGDRIWQIA LGSGFKCNSS VVVALRNVP STNNPWEQCL HKYPVEIDID
LKE

FIGURE 6

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EL3 1512 bases

CTACGTCAGG GTAGAACAAA GAGTAAACAC TTAAGCAAAA CAATTTGTCC TACTCTTAGG TTATCTCAA
 TGAAGAACCTT AAAGATGGTT TTCTTCAAGA TCCTCTTAT CTCTTTAATG GCAGGATTAG CCATGAAAGG
 ATCTAAGATC AACGTAGAAG ATCTCCAAA GTTCTCCCTC CACCATACAC AGAACACCT CCAAACCATA
 AGCCTTCTAT TGTTTCTTGT CGTTTTGTG TGGATCCTCT ACATGTTAAC CCGACCTAAA CCCGTTTACC
 TTGTTGATTT CTCTGCTAC CTTCCACCGT CGCATCTCAA GGTCAGTATC CAAACCTAA TGGGACACGC
 AAGACGTGCA AGAGAACGAG GCAATGTTG GAAGAACAAA GAGAGCGACC ATTTAGTTGA CTTCCAGGAG
 AAGATTCTTG AACGTTCCGG TCTTGGTCAA GAAACCTACA TCCCCGAGGG TCTTCAGTGC TTCCCACCTC
 AGCAAGGCAT GGGTGCTTCAG CGTAAAGAGA CGGAAGAAAGT AATCTTCCGA GCTCTTGACA ATCTTTTTCG
 CAACACCGGT GTAAAACCTG ATGATATCGG TATATTGGT GTGAATTCTA GCACGTTAA TCCAACCTCCA
 TCACTCGCCT CCATGATTGT GAACAAGTAC AAACTCAGAG ACAACATCAA GAGTTGAAT CTTGGAGGG
 TGGGTTGCAG TGCCGGAGTT ATAGCTGTTG ATGTCGCTAA GGGATTACTA CAAGTTCTATA GGAACACTTA
 TGCTATTGTA GTAAGCACAG AGAACATCAC TCAGAACTTA TACTTGGGA AAAACAAATC AATGCTAGTC
 ACAAACCTGTT TGTTCCCGT TGTTGGTGCCT GCGGTTCTGC TTTCAACAG ATCTAGAGAC CGTAACCGCG
 CCAAATACGA GCTTGTTCAC ACCGTACCGA TCCATACCGG ATCAGATGAT AGGTCTTGC AATGTGCGAC
 ACAAGAAGAG GATGAAGATG GTATAATTGG AGTTACCTTG ACAAAAGAATC TACCTATGGT GGCTGCAAGG
 ACTCTTAAGA TAAATATCGC AACTTGGGT CCTCTTGAC TTCCATTAAA AGAGAACGCTA GCCTTCTTTA
 TTACTTTTGT CAAGAAGAAG TATTTCAAGC CAGAGTTAAC GAATTATACA CCAGATTTCA AGCTTGCCCT
 TGAGCATTTC TGTATCCACG CTGGTGGAAAG AGCTCTAATA GATGAGCTGG AGAAGAACCT TAAGCTTTCT
 CCGTTACACG TAGAGCGTC AAGAATGACA CTACACAGGT TTGGTAACAC TTCTCTAGC TCAATCTGGT
 ACGAGTTAGC TTATACAGAA GCTAAAGGA GGATGAAGGA AGGAGATAG ATTGGCAGA TTGCTTTGGG
 GTCAGGTTTT AAGTGTAAACA GTTCAGTATG GGTGGCTCTG CGAGACGTTA AGCCTTCAGC TAACAGTCCA
 TGGGAAGACT GTATGGATAG ATATCCGGTT GAGATTGATA TT

EL3
FIGURE 7

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EL3 protein sequence
Molecular Weight 56801.10 Daltons
504 Amino Acids
66 Strongly Basic(+) Amino Acids (K,R)
48 Strongly Acidic(-) Amino Acids (D,E)
183 Hydrophobic Amino Acids (A,I,L,F,W,V)
127 Polar Amino Acids (N,C,Q,S,T,Y)
9.315 Isoelectric Point
19.797 Charge at PH 7.0

LRQGRTKSKH LSKTICPTLR LSPMKNLKMV FFKILFISLM AGLAMKGSKI NVEDLQKFSL HHTQNNLQTI
SLLLFLVVVFV WILYMLTRPK PVYLVDLSCY LPPSHLKVSQ QTLMGHARRA REAGMCWKNK ESDHLVDFQE
KILLERSGLGQ ETYIPEGLQC FPLQQGMGAS RKETEEVIFG ALDNLFRTG VKPDDIGILV VNSSTFNPTP
SLASMINVNKY KLRDNIKSLN LGGMGCSAGV IAVDVAKGLL QVHRNTYAIV VSTENITQNL YLGKNKSMLV
TNCLFRVGGAA AVLLSNRSRD RNRAKYELVH TVRIHTGSDD RSFECATQEE DEDGIIGVTL TKNLPMVAAR
TLKINIATLG PLVLPLKEKL AFFITFVKKK YFKPELRNYT PDFKLAFEHF CIHAGGRALI DELEKNLCLS
PLHVEASRMT LHRFGNTSSS SIWYELAYTE AKGRMKEGDR IWQIALGSGF KCNSSVWVAL RDVKPSANSP
WEDCMDRYPV EIDI

EL3
FIGURE 8

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EL4 cDNA 1650 bases

ATGGGTAGAT CCAACGAGCA AGATCTGCTC TCTACCGAGA TCGTTAACG TGGAATCGAA CCATCCGGTC
CTAACGCCGG CTCACCAACG TTCTCGGTTA GGGTCAGGAG ACGTTTGCCT GATTTCCTTC AGTCGGTGAA
CTTGAAGTAC GTGAAACTTG GTTACCACTA CCTCATAAAC CATCGGGTTT ATTTGGCGAC CATAACCGGT
CTTGTGCTGG TTTTAGTGC TGAGGTTGGG AGTTTAAGCA GAGAAGAGAT TTGGAAGAAG CTTTGGGACT
ATGATCTTGC AACTGTTATC GGATTCTCG GTGTCTTGT TTTAACCGCT TGTGTCTACT TCATGTCTCG
TCCCTGCTCT GTTTATCTTA TTGATTCGC TTGTTACAAG CCCTCCGATG AACACAAGGT GACAAAAGAA
GAGTTCATAG AACTAGCGAG AAAATCAGGG AAGTTCGACG AAGAGACACT CGGTTTCAAG AAGAGGATCT
TACAAGCCTC AGGCATAGGC GACGAGACAT ACGTCCCAG ATCCATCTCT TCATCAGAAA ACATAACAAAC
GATGAAAGAA GGTGTAAG AAGCCCTAC AGTGTATCTT GGAGCACTAG ACGAACTCTT CGAGAAGACA
CGTGTAAAAC CTAAAGACGT TGGTGTCTT GTGGTTAACT GTAGCATTT CAACCCGACA CCGTCGTTGT
CCGCAATGGT GATAAACCAT TACAAGATGA GAGGGAAACAT ACTTAGTTAC AACCTTGGAG GGATGGGATG
TTCGGCTGGA ATCATAGCTA TTGATCTTGC TCGTGACATG CTTCACTCTA ACCCTAATAG TTATGCTGTT
GTTGTGAGTA CTGAGATGGT TGGGTATAAT TGGTACGTGG GAAGTGACAA GTCAATGGTT ATACCTAATT
GTTTCTTTAG GATGGGTGT TCTGCCGTTA TGCTCTCTAA CGCTCGTGT GACTTTCGCC ATGCTAAGTA
CCGTCTCGAG CACATTGTCGACAACTCATAA GGCTGCTGAC GACCGTAGCT TCAGGAGTGT GTACCAGGAA
GAAGATGAAC AAGGATTCAA GGGGTTGAAG ATAAGTAGAG ACTTAATGGA AGTTGGAGGT GAAGCTCTCA
AGACAAACAT CACTACCTTA GGTCTCTTG TCCTACCTTT CTCCGAGCAG CTTCTCTTCT TTGCTGCTTT
GGTCCGCCGA ACATTCTCAC CTGCTGCCAA AACGTCCACA ACCACTTCCCT TCTCTACTTC CGCCACCGCA
AAAACCAATG GAATCAAGTC TTCCCTCTCC GATCTGTCCA AGCCATACAT CCCGGACTAC AAGCTCGCCT
TCGAGCATT TTGCTTCCAC GCGGCAAGCA AAGTACTGCT TGAAGAGCTT CAAAAGAATC TAGGCTTGAG
TGAAGAGAAAT ATGGAGGCTT CTAGGATGAC ACTTCACAGG TTTGGAAACA CTTCTAGCAG TGGAATCTGG
TATGAGTTGG CTTACATGGA GGCCAAGGAA AGTGTTCGTA GAGGGATAG GGTTGGCAG ATCGCTTTCG
GTTCTGGTTT TAAGTGTAAAC AGTGTGGTGT GGAAGGCAAT GAGGAAGGTG AAGAAGCCAA CCAGGAACAA
TCCCTGGGTG GATTGCATCA ACCGTTACCC TGTGCCTCTC

EL4
FIGURE 9

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EL4 protein sequence

Molecular Weight 61953.80 Daltons

550 Amino Acids

71 Strongly Basic(+) Amino Acids (K,R)

58 Strongly Acidic(-) Amino Acids (D,E)

191 Hydrophobic Amino Acids (A,I,L,F,W,V)

147 Polar Amino Acids (N,C,Q,S,T,Y)

9.036 Isoelectric Point

14.349 Charge at PH 7.0

MGRSNEQDLL STEIVNRGIE PSGPNAGSPT FSVRVRRRLP DFLQSVNLKY VKLGYHYLIN HAVYLATIPV
LVLVFSAEVG SLSREEIWKK LWVDYDLATVI GFFGVFVLTA CVYFMSRPRS VYLIDFACYK PSDEHKVTKE
EFIELARKSG KFDEETLGFK KRILQASGIG DETYVPRSIS SSENITTMKE GREEASTVIF GALDELFEKT
RVKPKDVGVL VVNCSIFNPT PSLSAMVINH YKMRGNILSY NLGGMGCSAG IIAIDLARDM LQSNPNSYAV
VVSTEMVGYN WYVGSDKSMV IPNCFFRMGC SAVMLSNNRRR DFRHAKYRLE HIVRTHKAAD DRSPRSVYQE
EDEQGFKGLK ISRDLMEVGG EALKTNITTL GPLVLPFSEQ LLFFAAALVRR TFSPAAKTST TTSPSTSATA
KTNGIKSSSS DLSPKYIPDY KLAFEHFCFH AASKVVLEEL QKNLGLSEEN MEASRMTLHR FGNTSSSGIW
YELAYMEAKE SVRRGDRVWQ IAEGSGFKCN SVVWKAMRKV KKPTRNNPVW DCINRYPVPL

EL4
FIGURE 10

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EL5 cDNA 1611 bases

TCGAGCTACG TCAGGGCTT TATATGCACA AATTCTCATA AAGTTTCAA TTTTATTCCA TTTTCTCGG
AAGCCATGGA AGCTGCTAAT GAGCTGTTA ATGGCGGATC CGTACAGATC CGAACAGAGA ACAACGAAAG
ACGAAAGCTT CCTAATTCT TACAAAGCGT CAACATGAAA TACGTCAAGC TAGTTTATCA TTACCTCATT
ACTCATCTCT TCAAGCTCTG TTGGTTCCA TTATGGCGG TTTTAGTCAC AGAGATCTCT CGATTAACAA
CAGACGATCT TTACCAGATT TGGCTTCATC TCCAATACAA TCTCGTTGCT TTCACTTTTC TCTCTGCTTT
AGCTATCTTT GGCTCCACCG TTTACATCAT GAGTCGTCCC AGATCTGTT ATCTCGTTGA TTACTCTTGT
TATCTTCCTC CGGAGAGTCT TCAGGTTAAG TATCAGAAAGT TTATGGATCA TTCTAAGTTG ATTGAAGATT
TCAATGAGTC ATCTTTAGAG TTTCAGAGGA AGATTCTTGA ACGTTCTGGT TTAGGAGAAG AGACTTATCT
CCCTGAAGCT TTACATTGTA TCCCTCCGAG GCCTACGATG ATGGCGGCTC GTGAGGAATC TGAGCAGGTA
ATGTTTGGTG CTCTTGATAA GCTTTTCGAG ATACCCAAGA TIAACCCATG GGATATTGGT GTGTTGGTTG
TGAATTGTAAG CTTGTTTAAT CCTACACCTT CGTTGTCAGC TATGATTGTT AACAAAGTATA AGCTTAGAGG
GAATGTTAAG AGTTTAACC TTGGTGAAT GGGGTTGAGT GCTGGTGTAA TCTCTATCGA TTTAGCTAAA
GATATGTTGC AAGTTCATAG GAATACTTAT GCTGTTGTGG TTAGTACTGA GAACATTACT CAGAATTGGT
ATTTTGGGAA TAAGAAGGCT ATGTTGATTG CGAATTGTTT GTTTCGTGTT GGTGGTTCGG CGATTTGGTT
GTCGAACAAG GGGAAAGATC GTAGACGGTC TAAGTATAAG CTTGTTCATA CCGTTAGGAC TCATAAAGGA
GCTGTTGAGA AGGCTTTCAA CTGTGTTTAC CAAGAGCAAG ATGATAATGG GAAGACCGGG GTTTCTGTTGT
CGAAAGATCT TATGGCTATA GCTGGGGAAG CTCTTAAGGC GAATATCCT ACTTTAGGTC CTTTGGTTCT
TCCTATAAGT GAGCAGATTC TGTTTTCAT GACTTTGGTT ACGAAGAAC TGTTTAACTC GAAGCTGAAG
CCGTATATTG CGGATTTCAA GCTTGCCTTT GATCATTCT GTATCCATGC TGGTGGTAGA GCTGTGATTG
ATGAGCTTGA GAAGAATCTG CAGCTTTCGC AGACTCATGT CGAGGCATCC AGAATGACAC TGCACAGATT
TGAAACACT TCTTCGAGCT CGATTTGGTA TGAACCTGGCT TACATAGAGG CTAAGGTAG GATGAAGAAA
GGAAACCGGG TTTGGCAGAT TGCTTTGGA AGTGGGTTTA AGTGTAAACAG TGCAGTTGG GTGGCTCTAA
ACAATGTCAA GCCTCGGTT AGTAGTCCGT GGGAACACTG CATCGACCGA TATCCGGTTA AGCTCGACTT
C

ELS
FIGURE 11

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EL5 protein sequence

Molecular Weight 60874.60 Daltons

537 Amino Acids

63 Strongly Basic(+) Amino Acids (K, R)

47 Strongly Acidic(-) Amino Acids (D, E)

198 Hydrophobic Amino Acids (A, I, L, F, W, V)

148 Polar Amino Acids (N, C, Q, S, T, Y)

9.107 Isoelectric Point

17.930 Charge at PH 7.0

SSYVRAFICT NSHKVFNFIP FFSEAMEAAAN EPVNGGSVQI RTENNERRKL PNFLQSVNMK YVKLGYHYLI
THLFKLCLVP LMAVLVTETIS RLTTDDLYQI WLHLQYNLVA FIFLSALAIF GSTVYIMSRP RSVYLVDYSC
YLPPESLQVK YQKFMDHSKL IEDFNESSLE FORKILERSG LGEETYLPQA LHCIPPRPTM MAAREESEQV
MFGALDKLFE NTKINPRDIG VLVVNCSLFN PTPSLSAMIV NKYKLRGNVK SFNLGGMGCS AGVISIDLAK
DMLQVHRNTY AVVVSTENIT QNWYFGNKKA MLIPNCLFRV GGSAILLSNK GKDRRRSKYK LVHTVRTHKG
AVEKAFNCVY QEQQDNGKTG VSLSKDLMAI AGEALKANIT TLGPLVLPIS EQILFFMTLV TKKLFNSKLK
PYIPDFKLA F DHFCIHAGGR AVIDELEKNL QLSQTHVEAS RMTLHRGNT SSSSIWYELA YIEAKGRMKK
GNRVWQIAFG SGFKCNSAVW VALNNVKPSV SSPWEHCIDR YPVKLD

EL5
FIGURE 12

EL6 1502 bases

TCTCCGACGATGCCCTCAGGCACCGATGCCAGAGTTCTAGCTCGGTGAAGCTCAAGTACGTGAAACTGGTTACCAA
TATTGGTTAACCATTTCTTGAGTTCTTTGATCCCAGTCATGGCTATTGTCGCCGTTGAGCTTCTCGGATGGGT
CTGAAGAGATCCTTAATGTTGAAATTCACTCCAGTTGACTAGTTCAAGGTTCTATGTTCTCCTCTTGTCACTC
TTCATCTCCACTGTTACTTCATGTCAGCCACGCACCATCACCTCGTTGACTATTCTTGTACAAGCCACCTGTC
ACGTGTCGTGTCCTCGAACCTTCAAGGACAAGCCTAAGAGCGTCGAGGTC
CAAATGAGAATCCTTGAAACGTTCTGCCCTCGGTGAGGAGACTTGTCCTCCGCTATTCAATTATATTCTCCACA
CCAACCATGGACGGCTAGAAGCGAGGCTCAGATGGTTATTCGAGGCCATGGACGATCTTCAAGAAAACGGGT
CTTAAACCTAAAGACGTCGACATCCTTATCGTCACCTGCTCTTTCTCCCACACCATCGCTCTCAGCTATGGTC
ATCAACAAATAAGCTTAGGAGTAATATCAAGAGCTTCATCCCAATTCAAATGCAATCATCGTCAGCACGGAGATCATAACCGCT
GTTGATCTAGCCCGCAGTTGCTCCAAGTTCACTCCGATGGGATGGGCTGAGCGCGGGCTGATCTCA
AATTACTATCAAGGCAACGAGAGGCCATGTTGTTACCCAATGTCCTCCGCATGGTGCAGCAGCCATACACATG
TCAAACCGCCGGCTGACCGGTGGCGAGGCAAATCAAGCTTCCCACCTCGTCCGGACACACCGTGGGCTGACGAC
AAGTCTTCTACTGTCATCGAACAGGAAGACACGGAGGAAAGGACACGTTGGCATTCAACTTGTCCAAGATCTCATGCC
ATCGCCGGTGAAGCCCTCAAGGCAAACATCACCAAACTAGTCTTGGTCTACCGGGCTGAGAACAAACTTCTCTTC
CTCACGTCCAATCGGACGTAAAATCTCAACCCGAAATGGAACCATACATACCGGATTCAAGCTGGCTTCGAA
CACTTTGCAATTACGCGAGGAGGCAGAGCGGTGATCGACGAGCTCCAAAAGAATCTACAACATACAGGAGAACACGTT
GAGGCCTCAAGAATGACACTACATCGTTTGTAACAGTCATCTCATGTTATGGTACGAGCTTAGCTACATCGAG
TCTAAAGGGAGAATGAGGAGAGGCATCGCTTGGCAAATCGCCTTGGAGGTGGTTCAAGTGTAACTCTGCCGTG
TGGAAGTGTAAACCGTACGATTAAGACACCTAACGGACGGACCAGGTGCGATTGATCGACCCTGTTACCCCTGTCTTATT
CCCGAAGTTGTCAAACTCTA

EL6
FIGURE 13

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EL6 protein sequence

Molecular Weight 56687.90 Daltons

500 Amino Acids

59 Strongly Basic(+) Amino Acids (K,R)

46 Strongly Acidic(-) Amino Acids (D,E)

182 Hydrophobic Amino Acids (A,I,L,F,W,V)

127 Polar Amino Acids (N,C,Q,S,T,Y)

8.909 Isoelectric Point

14.567 Charge at PH 7.0

SPTMPQAPMP EFSSSVKLKY VKLGYQYLVN HFLSFLLIPI MAIVAVELLR MGPEEILNVW NSLQFDLVQV
LCSSFFVIFI STVYFMSKPR TIYLVDYSCY KPPVTCRVPF ATFMEHSRLI LDKPKSVEF QMRILERSGL
GEETCLPPAI HYIPPTPTMD AARSEAQMVI FEAAMDLLFKK TGLKPKDVDI LIVNCRLFSP TPSLSAMVIN
KYKLRSNIKS FNLSGMGCSA GLISVDSLARD LLQVHPNSNA IIVSTEITP NYVQGNERAM LLPNCLFRMG
AAAIHMSNRR SDRWRAKYKL SHLVRTHRGA DDKSFYCIVYE QEDKEGHVGI NLSKDLMAIA GEALKANITT
IGPLVLPASE QLLFLTLSIG RKIFNPWKWP YIPDFKLAFE HFCIHAGGRA VIDEHQKNLQ LSGEHVEASR
MTLHRFGNTS SSSLWYELSY IESKGMRMRRG DRVWQIAFGS GFKCNSAVWK CNRTIKTPKD GPWSDCIDRY
PVFIPEVVKL

EL6
FIGURE 14

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EL7 1548 bases

ATGGACGGTCCGGAGAACATCAGACTCGGTGGTATGGTGGTGTGGTTCTGTTGGAGTCAGATCCGACAAACA
CGGATGCTACCGGATTCTCCAGAGCGTGAATCTCAAGTATGTGAAATTAGGTTACCATTACTTAATCTCAAATCTC
TTGACTCTCTGTTATTCCCTCTCGCCGTTATCTCGAAGGCTCTCAGATGAACCCAGATGATCTCAAACAG
CTCTGGATCCATCTACAATACAATCTGGTTAGTATCATCTGTTAGTCTCAGCGATTCTAGTCTCGGGTTAACGGTTAT
GTTATGACCCGACCTAGACCCGTTACTTGGTTGATTTCTCTTGTATCTCCCACCTGATCATCTCAAAGCtCCTTAC
GCTCGGTTCATGGAACATTCTAGACTACCGGAGATTCTGATGACTCTGCTCTCGAGTTCAACCGAAGATCCTTGAG
CGTTCTGGTTAGGGGAAGACACTTATGTCCTGAGCTATGCATTATGTTCCACCAGAGAATTCAATGGCTGCTGCT
AGAGAAGAAGCTAACAGTCATGTTGGTCTTAGATAACCTTTCTGTTATCTGCAATACAACTAATGTGAAACCAAGGATATT
GGAATCTTGTGTGAATTGTAGTCCTTAATCCAACCTCTGTTATCTGCAATGATTGTGAACAAGTATAAGCTT
AGAGGTAACATTAGAACAGCTACAATCTAGCGGTATGGTTGCGAGCGCGGGAGTTATCGTGTGGATCTTCTAAAGAC
ATGTTGTTGGTACATAGGAACACTTATGCGGTTGTTCTACTGAGAACATTACTCAGAAATTGGTATTTGGTAAC
AAGAAATCGATGTTGATACCGAAGTCTGTTGAGTTGGTGGCTCTGCGGTTTGCTATCGAACAGTCGAGGGAC
AAGAGACGGCTAACGTAAGTACAGGCTTGACATGTAGTCAGGACTCACCGTGGAGCAGATGATAAAAGCTTCCGTTGTGTT
TATCAAGAGCAGGATGATAACAGGGAGAACCGGGGTTTCGTTGCGAAAGATCTAACGGGATTCGAGGGAAACTCTC
AAAACCAATATCACTACATTGGGCTCTTGTCTACCGATAAGTGAGCAGATTCTCTTCTTATGACTCTAGTTGTG
AAGAAGCTTTAACGGTAAAGTGAACCGTATATCCGGATTCAAACTTGCTTTGAGCATTCTGTATCCATGCT
GGTGGAAAGAGCTGTGATCGATGAGTTAGAGAAGAATCTGCAGCTTCACCAAGTCTAGTCGAGGCTTCGAGGGATGACT
CTTCATCGATTTGGTAACACATCTCGAGCTCCATTGGTATGAAATTGGCTTACATTGAAGCGAAGGGAAAGGATGCGA
AGAGGTAATCGTGTGGCAAATCGCTTGGAGTGGATTAAATGTAATAGCGCGATTGGGAAGCATTAAAGGAT
GTGAAACCTTCGAACACAGTCCTGGAGATTGTATTGACAAGTATCCGTAACCTTAAGTTAT

EL7
FIGURE 15

EL7 protein sequence
Molecular Weight 57848.80 Daltons
516 Amino Acids
59 Strongly Basic(+) Amino Acids (K,R)
48 Strongly Acidic(-) Amino Acids (D,E)
189 Hydrophobic Amino Acids (A,I,L,F,W,V)
131 Polar Amino Acids (N,C,Q,S,T,Y)
8.872 Isoelectric Point
12.792 Charge at PH 7.0

MDGAGESRLG GDGGGDGSVG VQIRQTRMLP DFLQSVNLKY VKLGYHYLIS NLLTLCFLPL AVVISVEASQ
MNPDDLKQLW IHLOYNLVSI IICSAILVFG LTVYVMTRPR PVYLVDSCY LPPDHLKAPY ARFMEHSRLT
GDFDDDSALEF QRKILERSGL GEDTYVPEAM HYVPPRISMA AAREEAEQVM FGALDNLFAN TNVKPKDIGI
LVVNCNSLFNP TPSLSAMIVN KYKLGRGNIRS YNLGGGMGCSA GVIAVDLAKD MLLVHRNTYA VVVSTENITQ
NWYFGNKKSMLIPNCLFRVG GSAVLLSNKS RDKRRSKYRL VHVVVRTHRGADDKAFRCVYQ EQDDTGRTGV
SLSKDLMAIA GETLKTNITT LGPLVLPISE QILFFMTLVV KKLFNGKVKP YIPDFKLAFE HFCIHAGGRA
VIDELEKNLQ LSPVHVEASR MTLHCFGNTS SSSIWYELAY IEAKGRMRRG NRVWQIAFGS GFKCNSAIWE
ALRHVKPSNN SPWEDCIDKY PVTLSY

EL7
FIGURE 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11384

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A01H 5/00, C07H 21/00; C12N 15/00, 15/82
US CL : 800/205; 435/172.3; 536/23.6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/205; 435/172.3; 536/23.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/15387 A2 (CALGENE INC.) 08 June 1995, especially pages 57-71.	1,9,10,18-20,22-26, 28-30
Y		2-8,11-17,21,27
X	WO 96/13582 A2 (DNA PLANT TECHNOLOGY CORP.) 09 May 1996, especially pages 33-38.	1,9,10,18,19,24,25
Y		2-8,11-17,20-23,26-30

Further documents are listed in the continuation of Box C.

See patent family annex.

• Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"a"	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

07 AUGUST 1998

Date of mailing of the international search report

29 SEP 1998

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11384

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JAMES et al. Directed Tagging of the Arabidopsis FATTY ACID ELONGATION1 (FAE1) Gene with the Maize Transposon Activator. The Plant Cell. March 1995, Vol. 7, pages 309-319, see especially pages 316-317.	1,9,10 -----
Y		2-8,11,17-30